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NEW HELMINTH RECORDS FROM THE COTTON RAT, *SIGMODON HISPIDUS*, INCLUDING A NEW SPECIES, *STRONGYLOIDES SIGMODONTIS*

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The parasites of the cotton rat, *Sigmodon hispidus*, have been rather extensively studied, largely due to interest in a filarial parasite, *Litomosoides carinii*, which has been employed in chemotherapeutic and other studies relating to filarial worms in general. Baylis (1945) published a list of helminths recorded from this host, and Harkema and Kartman (1948) made a study of the helminths and ectoparasites of cotton rats in two localities in southeastern United States. The latter work, and probably that of Baylis also, was concerned with the southeastern subspecies, *Sigmodon hispidus hispidus*. The present study is concerned with helminths both from this subspecies and from the subspecies *S. h. texianus* in Texas. A new species, *Strongyloides sigmodontis*, is described, and three new host records, all spiruroids, are reported.

Following is a complete list of the helminths reported from cotton rats, including those here reported for the first time (marked with an asterisk):

Cestodes: *Monoecocestus sigmodontis* (Chandler and Suttles, 1922)
Andrya microti Hansen, 1947
Hymenolepis diminuta (Rudolphi, 1819)
Hymenolepis microstoma (Dujardin, 1845)
Raillietina bakeri Chandler, 1942
Taenia taeniaeformis (Batsch, 1786) larvae = (*Cysticercus fasciolaris* Rudolphi, 1808)

Nematodes: **Strongyloides sigmodontis*, n. sp.
Strongyloides sp. Baylis, 1945
Longistriata adunca Chandler, 1932
Trichostrongylus sigmodontis Baylis, 1945
Monodontus floridanus McIntosh, 1935
Physaloptera bispiculata Vaz and Pereira, 1935
Physaloptera muris-brasiliensis Diesing, 1861
Mastophorus muris ascaroides (Gmelin, 1790)
**Gongylonema* sp.
**Physocephalus sexalatus* (Molin, 1860)
**Rictularia ondatrae* Chandler, 1941
Litomosoides carinii (Travassos, 1920)

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Strongyloides sigmodontis, n. sp.

Examination of the feces of *Sigmodon hispidus texianus* from the Houston area showed rhabditiform larvae of *Strongyloides* in 14 of 20 animals examined. These larvae were found also in all of the 6 cotton rats obtained from Sarasota, Florida, belonging to the subspecies *Sigmodon hispidus hispidus,* which is presumably the same subspecies from which Baylis reported "*Strongyloides* sp." in 1945. It is probable that Baylis' species is the same as the new one here described.

Since identification of species of *Strongyloides* by morphological characters is very difficult if not impossible, as Augustine (1940) pointed out, a description of both the parasitic and free-living stages of the worm, and of the filariform larvae, is supplemented by experiments demonstrating host specificity.

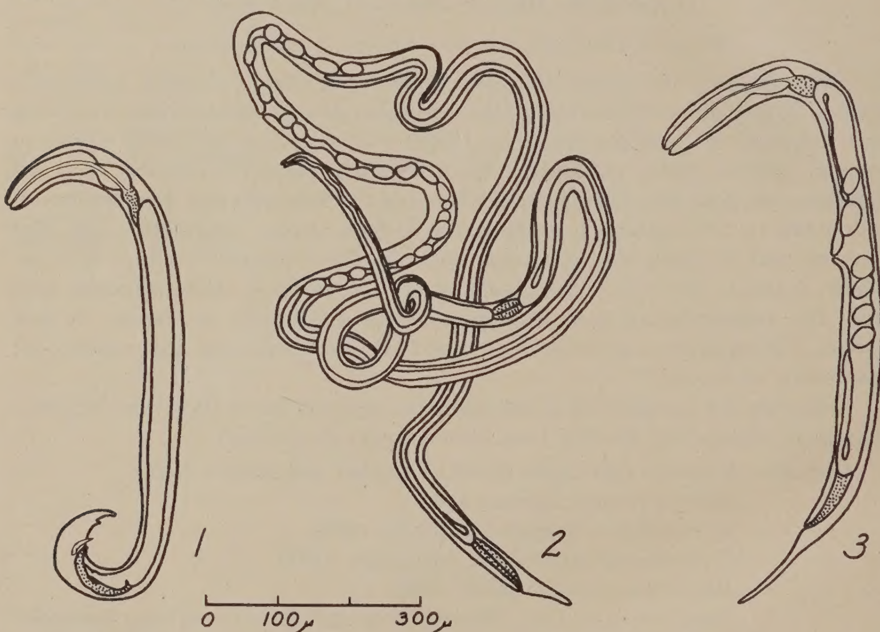

Fig. 1. Free-living male.

Fig. 2. Parasitic female.

Fig. 3. Free-living female.

Morphological Descriptions

Parasitic generation.

Parasitic females (Fig. 2) were obtained from the intestinal tract at autopsy by scraping the mucosa. Observations were made on both living and preserved material.

Length 3.9 to 4.7 mm.; diameter 23 μ in esophageal region, increasing to maximum of 31 μ shortly before anus. Diameter posterior to esophagus relatively uniform. Just posterior to anus, body abruptly narrowed, tail ending in a blunt, finger-like projection 53 μ long. Cuticle finely striated; mouth with 6 lips, opening into a shallow buccal cavity. Esophagus 870 μ to 900 μ long, comprising about 19% to 22% of total body length. Vulva with rather inconspicuous lips only slightly elevated, and located 2.6 to 2.8 mm. from anterior end, about 63% to 64% of

body length from anterior end. Anterior ovary turns backward just posterior to end of esophagus; posterior ovary turns forward just anterior to anus, which is situated 184 μ from end of tail. Both turns are of hairpin type, directly recurrent. Uteri with total of from 6 to 20 eggs, the latter measuring 50 μ × 27 μ. Eggs not fully developed, but apparently hatched before leaving host's body, as only rhabditiform larvae were found in feces.

Free-living generation.

Both males and females were obtained from fecal cultures.

Male (Fig. 1). Length from 880 μ to 896 μ, with maximum diameter of 46 μ to 48 μ in middle region of body. Width of body more or less uniform except in tail region. Esophagus 299 μ long. Testis occupies most of body cavity from just behind esophagus to anal opening. Spicules 34 μ long; tail curved ventrally, ending in rather blunt tip.

Female (Fig. 3). Body 668 μ to 984 μ long, the shorter specimens being immature. Width through middle of body from 50 μ to 60 μ. Esophagus 150 μ to 167 μ long, approximately one-fourth of body length. Vulva with rather conspicuous lips, located near middle of body. Uteri contain, on average, 8 to 9 well-developed eggs. Eggs about 46 μ × 31 μ. Loops of uteri turned with hair-pin type bends anteriorly near esophagus, and posteriorly just anterior to anus.

Filariform larvae. Length 651 μ to 660 μ; maximum width 16 μ to 21 μ. Esophagus slightly over 50% of total body length, measuring 373 μ to 378 μ. Tail tapered, with usual notched appearance. Anus 74 μ from posterior end.

Attempts were made to infect hamsters, guinea pigs, white rats, mice, and rabbits with subcutaneous injections of large numbers (1000–1200) of filariform larvae. No infections were established in these animals after 2–3 weeks, and it was concluded that this species is host-specific for the cotton rat.

A comparison of the parasitic female of *Strongyloides sigmodontis* with that of other species of *Strongyloides* reported from rodents is shown in the table. *S. papillosus* is included on the basis of Hall's (1916) report of its occurrence in *Rattus norvegicus* "and other rodents," although it is doubtful that these rodent parasites were actually *S. papillosus*. It will be seen that *S. sigmodontis* differs from *S. agoutii* Griffiths, 1940, in its slightly smaller size and lack of prominent vulvar lips, and in its failure to establish in the guinea pig after heavy doses. It differs from *S. ratti* Sandground, 1925, in size, its relatively shorter esophagus, and failure to infect white rats or mice. It differs from *S. robustus* Chandler, 1942, in being smaller with a slenderer body and longer esophagus. The uterine bends are of the hair-pin type rather than twisted as in *S. robustus*.

The free-living forms are identical morphologically with those of other species. From charcoal cultures of 72 hours, both filariform larvae and adults were obtained.

Rictularia ondatrae

From the intestinal tract of a cotton rat trapped on the Rice Institute campus, three female specimens of the genus *Rictularia* were obtained. Study of these specimens showed them to be identical with *R. ondatrae* Chandler, 1941, reported by that author from the muskrat in East Texas. This infection undoubtedly was accidental, and its occurrence in the cotton rat (*Sigmodon hispidus texianus*) is reported here as a new host record.

Physocephalus sexalatus

Several specimens of a species of *Physocephalus* were obtained by Mr. William Hegener of Sarasota, Florida, from a cotton rat obtained in that general area. These worms proved to be *P. sexalatus,* a common parasite of pigs. They con-

	S. papillosus	*S. robustus*	*S. ratti*	*S. agoutii*	*S. sigmodontis*
Length of parasitic female.	3.5–6 mm.	4.5–6.8 mm.	1.85–3.03 mm.	3.9–6.4 mm.	3.9–4.7 mm.
Length of esophagus.	.65–.80 mm.	.86–1.26 mm. (17.7–19%)	.55–.78 mm. (22.2–33.0%)	.9–1.75 mm. (20–25%)	.87–.9 mm. (19.1–21.9%)
Shape of tail.	Constricted just behind anus. Ends bluntly.		Finely tapered.	Constricted just behind anus, ends in finger-like projections.	Constricted just behind anus. Ends bluntly.
Vulva.	60–63% of body length from anterior.	57–65% of body from anterior.	1.27–1.73 mm. from anterior end.	1.27–2.3 mm. from posterior end. Has prominent lips.	63–64% length of body from anterior end. Lips rather inconspicuous.
Loops of ovaries.	Twisted.	Usually twisted but less than *S. papillosus*.	Directly recurrent.	Recurrent or twisted. Varies.	Directly recurrent.
Lips of mouth opening.	6	6	6	3, with 2 papillae on each.	6
Eggs.	40–60 $\mu \times$ 20–25 μ	55–60 $\mu \times$ 27–30 μ	42–52 $\mu \times$ 28–31 μ	46–58 $\mu \times$ 23–29 μ	49.0–51 $\mu \times$ 26–29 μ
Host.	Sheep; will establish in rabbits.	Tree-squirrels.	Norway rat; will establish in white rats.	Agouti; will establish in guinea pig.	Cotton rat.
Distribution	Cosmopolitan	East Texas	Cosmopolitan	Trinidad	Southern U.S.A.

formed with textbook descriptions of this worm with respect to size, lateral alae, cervical papillae, and the tail of the male. However, detailed examination revealed differences which at first appeared to be of possible specific value. Descriptions of *P. sexalatus* indicate a pharynx length of 230 μ to 280 μ, a pronounced cervical swelling terminating abruptly about 230 μ from the anterior end, or at the level of the end of the pharynx, and three lateral alae starting at this point. Specimens from the cotton rat showed the pharynx to be shorter, 183 μ to 200 μ, with the lateral alae beginning 60 μ to 80 μ posterior to its termination. No pronounced cervical swelling was observed. The vulva varied in position from the anterior third to the posterior third of the body. Railliet and Henry (1911) reported the vulva of *P. sexalatus* as being located at the junction of the third and fourth fifths, while Seurat (1912) reported it at the anterior third of the body. Apparently this structure is variable. Examination of specimens of *P. sexalatus* from pigs, kindly supplied by Dr. A. D. Turk of Texas A. and M. College, revealed one specimen, measuring 12 mm. without a pronounced cervical swelling, a pharynx only 180 μ long, and the lateral alae beginning 33 μ posterior to the posterior end of the pharynx. It would seem, then, that such morphological characters as the cervical swelling, length of pharynx, and position of vulva are very variable and not of specific value.

From these considerations, it appears doubtful that all of the described species of *Physocephalus* are valid; some may prove to be synonyms of *P. sexalatus*, which has a worldwide distribution. The other species assigned to the genus are: *P. leptocephalus* (Rudolphi, 1819) from *Bradypus tridactylus; P. gracilis* (Rudolphi, 1819) from *Bradypus tridactylus; P. mediospiralis* (Molin, 1860) from *Dasyprocta agouti; P. cristatus* (Seurat, 1912) Railliet, 1915, from dromedary and ass; and *P. lassancei* Travassos, 1921.

Among specific characters given are size, length of the pharynx, cuticular swelling, position of the vulva in the female, and number of papillae on the tail of the male. All of these with the possible exception of the last, are so variable, as indicated by specimens of *P. sexalatus*, as to be valueless for specific identification. *P. cristatus* is distinguished by the presence of longitudinal crests on the head, and the fact that the whole body of the male is turned at an angle of 180° instead of having the tail rolled into a spiral. The two species from sloths are separable from each other and from other members of the genus only by size, length of esophagus, size of the spicules, and number of papillae on the male tail (Neiva et al, 1914). Whether the male differential characters are valid cannot be determined with certainty without further study. *P. mediospiralis* is too imperfectly known to permit one to be sure even that it belongs to the genus *Physocephalus*. The other species, *P. lassancei,* has since been removed from the genus by Cuocolo (1943) and placed in the genus *Pereiraia.* At present, therefore, there seems to be sound evidence for only two species in the genus. The ability of *P. sexalatus* to establish itself in a rodent as well as in pigs, tapirs, and equines shows a wide versatility with respect to hosts.

Gongylonema sp.

A species of *Gongylonema* was obtained from a nodule in the stomach wall of a cotton rat from the Sarasota, Florida, area. Only two incomplete female specimens were obtained. The body measures 320 μ in width. The esophagus is ap-

proximately 7 mm. long, with the anterior portion 800 μ long. These measurements correspond to reported measurements of *G. neoplasticum* (Fibiger and Ditlevsen, 1914) found in rats, and of *G. pulchrum* Molin, 1857, found in a large variety of animals, including pigs, cattle, camels, horses, man, bears, rats, guinea pigs, and rabbits. These species are separated only by differences in the males. Specific identification, therefore, was not possible.

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DYNATOCHELA PRIMUS N. SP., N. GEN. (ACARINA; LAELAPTIDAE; IPHIOPSINAE)

Hugh L. Keegan[1]

Mites of subfamily *Iphiopsinae* Kramer, 1886 are distinguished from other *Laelaptidae* in that the stigmata are almost without peritremes. The species described have been found as parasites and commensals of insects and myriapods. Vitzthum (1942) included three genera in the subfamily, and separated them in the following manner:

1. All tarsi with claws:
   Berlesia Canestrini, 1884
2. Tarsus I without claws, tarsi II, III, and IV with claws:
   Iphiopsis Berlese, 1882
3. All tarsi without claws:
   Jacobsonia Berlese, 1910

The species here described possesses claws on all tarsi, but differs from *Berlesia* in that both chelae are toothed. A new genus *Dynatochela* is therefore proposed to include this new species, *Dynatochela primus,* which is designated as the genotype. The generic name was chosen because of the stout, massive, structure of the chelae of both sexes.

Mesostigmata Canestrini, 1891
  Laelaptidae Berlese, 1892
    Iphiopsinae Kramer, 1886
      Dynatochela gen. nov.

All tarsi with claws and pulvilli; both chelae with teeth; anal shield of male separate; one species, the genotype, found on a beetle, *Phyllophaga* sp., at Omaha, Nebraska.

Genotype: *Dynatochela primus* n. sp.

Female: Dorsal shield very difficult to trace; setae of shield relatively short and smooth, and number 17 in female specimen examined. Dorsal body setae short and smooth; are larger at body margins. Lacinae of tritosternum barbed. Presternal area indistinct, not sculptured. Sternal shield not heavily chitinized; the three pairs of sternal setae are short, stout, and smooth, and distinctly larger than those of dorsal shield; sternal pores short, with rounded ends; margins of shield indistinct. Genitoventral shield short, with a rounded posterior margin, and with one pair of genitoventral setae, which are short, stout, and smooth, and are located near the posterior margin of the shield. Outline of anal shield not distinct on the only available specimen; only two anal setae are visible. Endopodal and metapodal shields not present. Metasternal setae distinctly smaller than sternal setae; about equal in size with genitoventral setae. Ventral body setae of about equal length with setae of dorsal shield. Peritreme very short, about two and one half times as long as it is wide. Epistome not clear in specimen examined. Chelae relatively stout. Fixed chela with a bifid tip and a large distal tooth, as well as a distal seta; bears two proximal tufts of setae. Movable chela the smaller of the two; bears one blunt tooth. Maxillary corniculi distinct; all maxillary setae smooth. All palpal setae smooth; ventrally located coxal seta is largest on palp; tarsus bears the usual bifurcate seta of the *Laelaptidae.* Legs relatively short and stout. Most setae on legs small, although some ventral setae, especially on coxae and tarsi, are stout and spur-like; two conspicuous spur-like setae on femur of leg I; all setae smooth.

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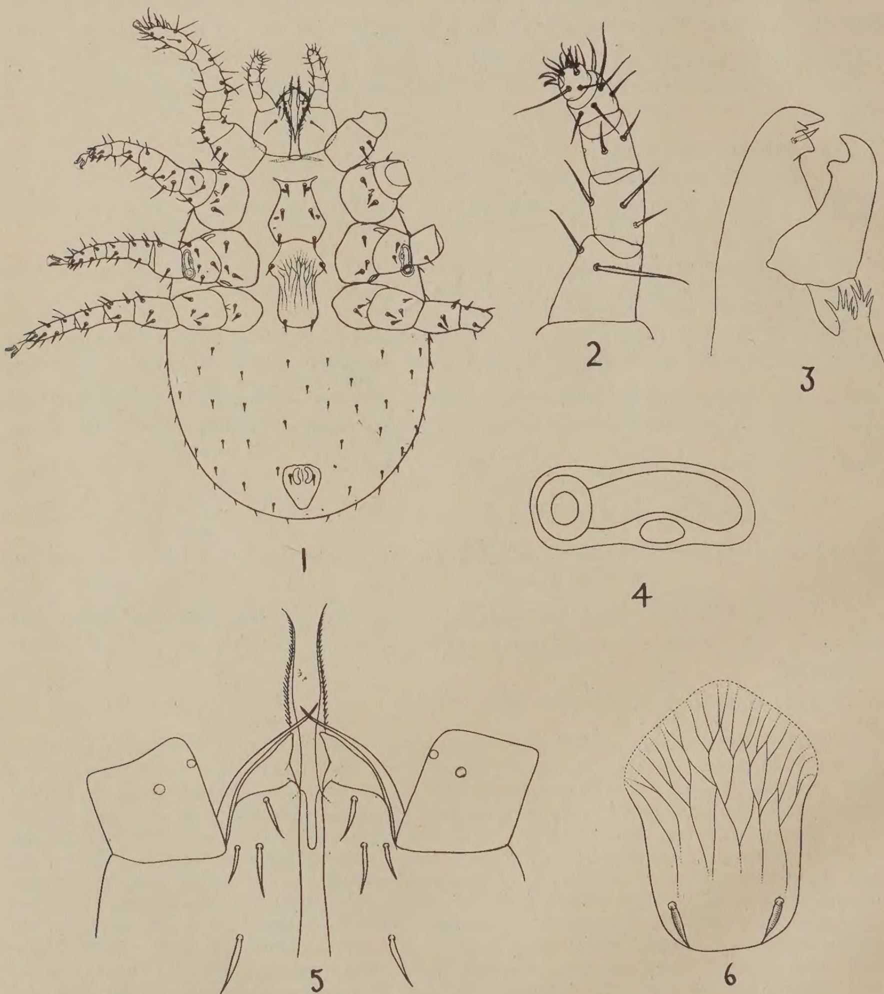
A foramen near antero-distal margin of coxa I. Length-width ratios of tarsi are: tarsus I 3.5 : 1, tarsus II 3.5 : 1, tarsus III 5 : 1, and tarsus IV 5 : 1. The relatively short legs, spur-like ventral setae on coxa and tarsus of each leg, and short, slender, dorsal setae are distinctive.

Male: Dorsal shield more heavily chitinized than in female, but with a similar pattern of setation. Posterior to shield are about twenty small, irregular shields, which appear to be in the cuticle rather than at its surface. Tritosternum as in female. Ventral setation much as in female, but with only one seta in the genitoventral region of the available specimen. Posterior

PLATE I



EXPLANATION OF PLATE I

Dynatochela primus sp. nov., female

FIG. 1. Ventral view.
FIG. 2. Palp.
FIG. 3. Chelicera.
FIG. 4. Peritreme.
FIG. 5. Ventral view of gnathosome showing corniculi and setae.
FIG. 6. Genitoventral shield.

margin of ventral shield rounded; shield is not sculptured. Anal shield, peritreme, and ventral body setae as in female. Chelae show sexual dimorphism. Fixed chela distinctly humped; its tooth and distal seta are as in female. Movable chela with a sperm carrier arising about midway on its length; its tooth is more pointed than that of female. Maxillary corniculi possess blunt tips. Palpi as in female. Leg I distinctly more slender than others. Femur, genu, and tibia of leg II each with a heavy, blunt, ventral spur. Tarsus of each of the three posterior pairs of legs with a short, terminal spur. Tibia of leg IV possesses a stout, blunt spur; other setation of all legs as in female.

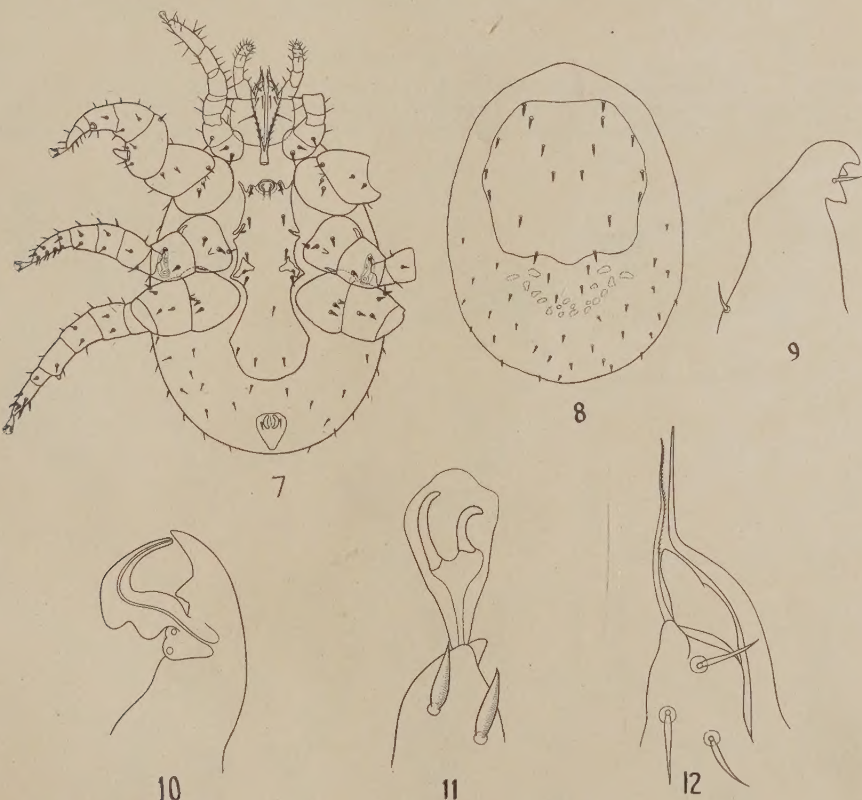
No larvae or nymphs were found.

Type: Female specimen taken from a beetle, *Phyllophaga* sp., at Omaha, Nebraska, 17 June, 1945, by R. Pegau.

Paratype: Male specimen, same data as type. Both type and paratype have been deposited the collections of the United States National Museum.

It is unfortunate that both type and paratype are so distorted that it is impossible

Plate II



Explanation of Plate II

Dynatochela primus sp. nov., male

Fig. 7. Ventral view.
Fig. 8. Dorsal view.
Fig. 9. Fixed chela.
Fig. 10. Movable chela.
Fig. 11. Distal end of tarsus of leg III.
Fig. 12. Maxillary cornicula.

to obtain accurate measurements of body length and width. For the same reason the posterior portion of the anal shield cannot be seen, and only two anal setae are visible, although it is probable that a third is present as in other *Laelaptidae*.

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A NEW SPECIES OF PARASITIC COPEPOD FROM THE ANGEL SHARK

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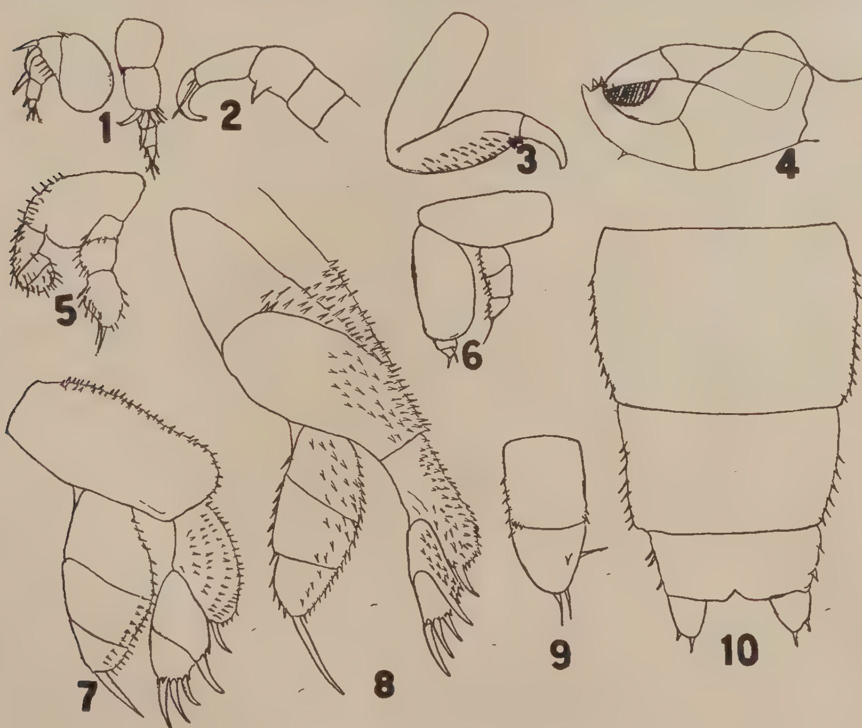
Through the courtesy of Clinton E. Atkinson, U. S. Fish and Wildlife Service, the writer was permitted to cruise off the North Carolina coast on the M. V. Albatross III, February 14–21, 1950. On February 15 an angel shark, *Squatina dumeril* Le Sueur, was taken in a trawl at a depth of 216 ft., 18 miles off Cape Lookout. From the gills of this fish 6 females of the parasitic copepod that is described in this paper were taken.

Type No. 90723, U. S. National Museum.

Eudactylina spinula, n. sp.

Body generally spinulose on dorsum, along the margins, and on swimming legs. Cephalon wider at the posterior end, angles rounded at both ends. First segment of thorax ¼ as long as second and narrower. Segments 2 to 4 progressively longer and fourth segment widest.



Figs. 1–10. *Eudactylina spinula* n. sp., female. 1, two views of first antenna; 2, second antenna; 3, second maxilla; 4, maxilliped; 5, first leg; 6, second leg; 7, fourth leg; 8, third leg; 9, fifth leg; 10, abdomen.

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Genital segment nearly half as long as fourth and as wide. Sutures between segments 3, 4, and 5 poorly defined. Abdomen 3-segmented, the segments all wider than long, decreasing in length posteriorly. Egg strings more than half as long as body, eggs in a lineal series; 15 to 17 in a string, half as wide as genital segment.

Antenna 1 (Fig. 1) 5-segmented; first and second segments about equal in length; segments 3–5 progressively shorter; segment 1 with a short spine; segment 2 with two strong spines, one straight and the other curved, and 3 setae; segments 3 and 4 with short, stout spines; segment 5 with three terminal setae. Second antenna (Fig. 2) 4-segmented; segment 3 with a strong lateral spine; segment 4 with a slender terminal spine and a strong curved terminal claw. Second maxilla (Fig. 3) 2-segmented; segment 2 spinulose on posterior margin; terminal claw smooth and curved. Maxillipeds (Fig. 4) chelate; the fixed claw ends in 3 short spines and has a small spine on the posterior margin.

First four swimming legs all with 2 rami which are 3-segmented; more or less spinulose on posterior margins. First leg (Fig. 5) setose, outer ramus shorter than inner; the latter with two long terminal setae. Second leg (Fig. 6) with first segment of lateral ramus very long, smooth; with two terminal setae; median ramus spinulose on lateral margin. Third leg (Fig. 8) very spinulose; outer ramus with 5 strong hooks, inner ramus with one. Fourth leg (Fig. 7) shorter than leg 3, with similar armature. Fifth leg (Fig. 9), 2-segmented, terminal segment with 2 terminal and one lateral setae and a short ventral spine, spinulose on margins. Furcula with two terminal spines.

Male—Unknown.

This species differs from Bere's (1936) *E. turgipes,* in the character of the second leg, and the terminal segment of the movable digit of the maxilliped, from her *E. longispina* in armature of legs 1, 2, and 4, and from her *E. squamosa* in armature and proportions of the legs and the maxilliped.. It differs from Heller's (1865) *E. aspera* in legs 1, 2, maxillipeds, and furcula. From Wilson's (1932) *E. spinifera* it differs in antennae 1 and 2, legs 2, and 5. From Hesse's (1883) *E. squatinae angeli* it differs in having short spines instead of slender setae on the body and appendages. The first antenna is 5-segmented, instead of 6; the second antenna bears a strong spine on the inside of segment 3 and segment 4 has a slender spine and a curved terminal hook; maxilla with a fixed claw that ends in 3 short spinulose processes and bears a short spine on the posterior margin. The swimming legs differ in proportions and armature. The abdomen is 3-segmented. The egg strings are shorter and contain fewer eggs. It is named for the spinulose exterior.

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OBSERVATIONS ON THE AMYLOLYTIC ACTIVITY OF *ENTAMOEBA TERRAPINAE**

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The enrichment of cultures of most species of entozoic amoebae by addition of starch has been common practice ever since the original observations of Dobell and Laidlaw (1926). But in nutritional studies the indiscriminate use of ground rice introduces a wide variety of uncontrollable factors. Polished rice contains appreciable amounts of protein, nucleoprotein and lipids in addition to its carbohydrate components, and whole rice will add to these a variety of vitamins and other possible growth supplements. Physical purification by the methods described by Griffin and McCarten (1949a) should eliminate the inert and protein components of the original rice, but the remaining starch grains are still complexes of at least three components: the linear carbohydrate usually referred to as "amylose" with its adsorbed lipids (cf. Schoch, 1945), and the branched "amylopectin." Aside from the stimulating effect of the unsaturated fatty acids as shown by Griffin and McCarten (1949b), there remains the question of the comparative nutritional efficiency of the two types of carbohydrate.

There is no reason to doubt the independent assimilation of starch by such entozoic amoebae as *Endamoeba histolytica* and its close biological relative *Entamoeba terrapinae*. We have reported one series of experiments in which fair growth of amoebae was obtained in monobacterial association with an organism which was incapable of utilizing starch (Griffin and McCarten, 1950a), and the liver-cholesterol-broth now used in our work fails to support growth of species of the genus *Endamoeba* in the absence of particulate starch. However, it is not clear whether the amoebae make use of the entire starch grain with uniform efficiency or have a predilection for one or the other carbohydrate components. Nor is there any evidence as to the nature of the mechanism of amyloclasis.

We have, therefore, followed the amyloclastic activity of several substrains of *Entamoeba terrapinae* by means of the method of Smith and Roe (1949) for the determination of alpha-amylase. Although the method was designed for the detection of this enzyme in blood and urine, where it is the only amyloclastic enzyme present, the procedure will detect any amyloclastic system capable of attacking the linear component of starch.

As substrates for the cultures we have used normal rice starch which, according to Bates *et al* (1943) contains at least 17% amylose and about 0.5–0.6% lipids (Schoch, 1942), and starch prepared from waxy rice[1] which is composed entirely of amylopectin and therefore contains no appreciable lipids.

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[1] The authors are indebted to Jenkin W. Jones, Bureau of Plant Industry, U.S.D.A., for the sample of waxy rice.

MATERIALS AND METHODS

Four substrains of our (165) strain of *Entamoeba terrapinae* were established with different bacterial floras. The flora of substrain (100) was a mixture of four known strains of bacteria isolated from the parent culture and constituted the minimum mixture which would give all of the manifestations of amoebic development. Substrain (227) was derived from the original strain (165), while (2) and (A) were the result of contaminations of (227) which appeared favorable to amoebic growth. All cultures were maintained by transfer of 100,000 amoebae at 48 hr intervals with incubation at 30° C.

Starches were prepared from normal and waxy rices by the methods previously described (Griffin and McCarten, 1949a). They were added to the cultures in quantities sufficient to give active growth throughout the incubation period with a minimal residue at the time of testing and transfer.

Amoebic yields were determined by direct counts in a Fuchs-Rosenthal chamber of 0.2 mm. depth. Amyloclastic activity was determined on an aliquot of each culture and expressed as "amylase units" per 100 ml. as defined by Smith and Roe (1949).

TABLE 1.—*Analysis of Variance and Covariance*

Source of Variation	df	Variances		Errors of Estimate	
		Yields	Amylase	df	Variance
Varieties of starch	1	133,036*	8,507*	1	1,738
Floras	3	150,003*	18,832*	3	8,278*
Interaction	3	23,522*	2,633*	3	1,395
Error	40	2,693	517	39	512

* Highly significant, probability less than 0.01.

The experiment was factorial and carried through six serial replications. Serialization, rather than simultaneous replications, was adopted to compensate for chronological variation. By this device periodicity of behavior is included as a part of the uncontrollable error (cf. Griffin and McCarten, 1950b) giving a more rigorous basis for judgment of the significance of other effects.

EXPERIMENTAL RESULTS

Application of the method of analysis of variance and covariance (Snedecor, 1946) to the observed data results in the statistics of Table 1. At this stage the salient feature of the analysis is the high degree of correlation existing between amylase units and yield. For the entire experiment the variance ratio (F) for regression of amylase units on yield was 76.63; df = 1, 46. The probability of random occurrence is far less than 0.001. When the separate components of the analysis are taken into account there is no significant departure from regression (as judged by the "Errors of Estimate") other than that associated with the bacterial floras. In this case the variance is highly significant ($F = 16.17$; df = 3, 39). The appropriate factor for adjusting the observed amylase units is therefore the regression within floras with b = 0.198.

After adjustment of the amylase units the results on which our judgment is to be based are as displayed in Table 2. Here the individual components are assayed by means of the variance ratio (F) appropriate to the particular effect involved. As was indicated by the initial analysis of variance, the mean differences

in yield between varieties of starch and among bacterial floras are most unlikely to have been fortuitous. Even more meaningful are the interaction and the individual comparisons among floras. The variety-flora interaction is, of course, a measure of the failure of the single differences to be consistent. Thus the mean variety difference in yield, 231.3, with flora (2) stands in sharp contrast to the insignificant difference, 47.7, with flora (227). With normal rice starch as the substrate there is no possible set of orthogonal comparisons which will indicate homogeneity of any pair of floras; i.e., each differs significantly from all the others. When waxy rice starch is the substrate, however, the only highly significant mean difference is that between the yield from flora (100) and the others. This difference, 117.2, has a variance ratio (F) of 29.55; df = 1, 20. Floras (2) and (A) give essentially equal yields ($F = 0.23$; df = 1.20), and the mean difference between flora (227) and the average of (2) and (A), 66.0, is of dubious significance. The variance ratio of the latter ($F = 6.07$; df = 1, 20) has a probability of 0.025. With

TABLE 2.—*Yields and Amyloclastic Activity of Cultures of* E. terrapinae
Mean yields of six serial cultures

	Varieties of Starch	Floras 100	227	2	A	Mean	F
Yields per culture $\times 10^{-4}$	Normal	159.7	261.0	513.0	379.5	328.3	55.44**
	Waxy	120.0	213.3	281.7	277.0	223.0	11.88**
	Mean	139.8	237.2	397.3	328.2	275.6	55.70**
	Difference	39.7	47.7	231.3	102.5	105.3	
	F	6.45*	1.84	54.37**	9.32**	49.40**	
Variety-flora interaction: $F = 8.73$**							
Adjusted amylase units per 100 ml	Normal	109.3	137.8	160.5	104.7	128.1	5.77**
	Waxy	96.8	130.5	137.2	124.3	122.2	5.58**
	Mean	103.1	134.2	148.8	114.6	125.1	9.31**
	Difference	12.5	7.3	23.3	−19.6	5.9	
	F	1.43	0.29	1.65	4.69	0.76	
Variety-flora interaction: $F = 1.88$							

Amylase units adjusted for covariance within floras, $b = 0.198$.
* Significant, probability 0.05–0.01.
** Highly significant, probability less than 0.01.

47 possible individual comparisons we would expect, on the average, that one in 40 would have a variance ratio of this magnitude in random sampling. For this and other reasons we are inclined to regard the yields from waxy rice starch as essentially homogeneous with floras (227), (2) and (A).

In the case of the adjusted values for amylase, the varietal differences and interaction are notably homogeneous in the statistical sense. This is, of course, to be expected since the analysis of covariance showed no significant departures from the regression within floras which was the basis for the adjustment. When we turn to the individual comparisons we find that with normal rice starch as the substrate floras (100) and (A) have essentially the same effect on amylase production by the amoebae as do floras (227) and (2). The mean difference between the latter is 22.7 units, a non-significant difference ($F = 2.04$; df = 1, 20). Thus there are factors affecting amoebic yield which are not operative with respect to amyloclastic activity. The mean difference between these two pairs of floras is 42.2 units which is highly significant ($F = 14.15$; df = 1, 20). Thus the variability among floras for normal rice starch is, as far as significance is concerned, entirely due to this difference. With waxy rice starch as the substrate the only significant difference in amyloclastic activity lies between cultures with flora (100) and the

remaining three. The mean difference of 33.9 units is highly significant, ($F = 16.25$; df = 1, 20), whereas the differences among the other three floras are less than average ($F = 0.78$; df = 2, 20).

Concomitant with the above observations we have repeatedly carried amoeba-free cultures of the bacterial floras through all of the procedures with both starch substrates and have been unable to demonstrate appreciable amyloclastic activity under identical conditions.

DISCUSSION

The most efficient statistical analysis and the most rigorous criteria of judgment can do no more than call attention to the random probability of occurrence of a difference. The ultimate causes of an observed difference must depend on our ability to detect and test the effect of common and divergent factors.

The production of amyloclastic enzymes by *Entamoeba terrapinae* is obviously a complex of such common and divergent factors. The high correlation existing between amylase units and numerical yields testifies to independence of action on the part of the amoebae as does the failure of the bacterial floras to show amlyoclastic activity. Such independence is not absolute, however, since the composition of the bacterial flora introduces differences of significant magnitude. Similarly, although the amyloclastic activity is independent of the substrate for any given flora, the effects of the floras within a given substrate are different. One need only recall the pairing of floras (100) and (A) and floras (227) and (2) with normal rice starch as the substrate as contrasted with the homogeneous nature of floras (227), (2) and (A) when substrate is waxy rice starch to gain an insight into the complexity of the situation. It is quite obvious that resolution of these floral differences must await bacteriological analysis and determination of the contribution of the individual species of bacteria.

The situation is further complicated by the probability that we are not dealing with a single amyloclastic enzyme. Our use of "amylase units" to express the degree of amyloclastic activity is purely a convenience of definition and should not necessarily be interpreted as meaning that the amoebae are elaborating a true "amylase." We have reason to suspect the presence of at least two amyloclastic enzymes since, among other things, reduction of the phosphorus content of the medium to minimal levels markedly affects the type of amyloclastic activity.

The observed differences among yields of amoebae are even more complex since the varietal differences were, for the most part, significant. They are, however, partially interpretable in the light of existing knowledge as to the composition of starches and with respect to as yet unpublished observations of our own. We earlier called attention to the fact that normal rice starch contains an appreciable amount of lipid adsorbed on its amylose moiety. A large part of this is unsaturated fatty acid which, as we have shown, has a powerful stimulating effect on amoebic growth. On removal of the lipid by the methods recommended by Schoch (1942) there is a reduction of about 45% in numbers of amoebae whereas the yields from waxy rice starch are unaffected when it is similarly treated. The latter, being amylose-free, contains no appreciable lipid. Such "defatted" normal rice starch can be fully restored nutritionally by impregnation with oleic acid. In comparison with waxy rice starch, "defatted" normal rice starch is deficient by

about 20% with respect to yield of amoebae. If it be coincidence it is indeed striking that this is close to the probable amylose content of normal rice starch. The value given by Bates *et al* (1943) was 17% amylose which, by comparison with the later observations of Schoch and Williams (1944), is probably several percent low. As an hypothesis for further testing one might assume that, for some reason, amylose is without nutritive value to the amoebic and that they utilize only the amylopectin moiety of starch as an energy source. There are certain technical difficulties in offering purified amylose and amylopectin to the amoebae as the sole carbohydrate source, but incomplete data suggest that the amoebae do indeed make independent use only of the amylopectin.

At least a part of the flora-induced variations may well be associated with the lipid of normal rice starch. It will be recalled that when waxy rice starch was the substrate the yields with floras (227), (2) and (A) were nearly homogeneous in contrast to their complete heterogeneity with normal rice starch. Unfortunately aside from isolated observations of stimulating effects of oleic acid on the growth of a few species of bacteria and inhibitory action with others we have no information as to its general activity and no knowledge at all as to its role in metabolism. It is therefore impossible, at the moment, to do more than call attention to the observed effects.

Although the above studies were carried out in a medium of indeterminate chemical composition, it should be obvious that investigations involving chemically controlled media should take cognizance of the complex nutritional nature of the starch grain. Under the preparative conditions used herein waxy rice starch should constitute practically pure usable carbohydrate. We must admit, however, that our inquiries into the presence of nitrogenous compounds are still incomplete. But since waxy rice is not generally available, exhaustively defatted normal rice starch should be essentially equivalent. If one wishes to take advantage of the stimulating properties of oleic acid, the defatted normal rice starch may be impregnated with the pure fatty acid by techniques which are by no means formidable.

SUMMARY

1. Cultures of *Entamoeba terrapinae* produce amyloclastic enzymes independently of but conditioned by the associated bacterial flora.

2. Amyloclastic activity is the same regardless of whether the substrate in the culture is starch from normal or waxy rice.

3. It is suggested that differences in yield of amoebae from normal and waxy rice starches is a function of the lipid content and nutritional unavailability of the amylose moiety of the former.

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OBSERVATIONS ON CERTAIN PHASES OF NUTRITION AND HOST-PARASITE RELATIONS OF *HYMENOLEPIS DIMINUTA* IN WHITE RATS

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In a series of papers emanating from this laboratory (Chandler, 1939, 1943; Addis and Chandler, 1944, 1946) certain conclusions were reached concerning the host-parasite relations of *Hymenolepis diminuta* in white rats, particularly with reference to their nutrition. The evidence indicated that whereas the worms are dependent upon the food of the host for the large amounts of carbohydrate required they are able to obtain adequate amounts of nitrogenous foods directly from the host. They were also found to be independent of alimentary vitamins with the exception of some unidentified substance associated with the B-complex in brewer's yeast, the need for which is influenced by the sex hormones.

In the present paper some of these experiments have been repeated and extended, using more adequately controlled experimental diets, to determine whether *H. diminuta* obtains its vitamins from the host, from bacterial synthesis in the intestine, or by its own synthesis. For this purpose thiamin was selected for experimentation, since (1) it is a typical vitamin; (2) as far as known, it is needed in the metabolism of all kinds of animals; (3) good quantitative methods for its determination are available; and (4) its preparation with radioactive sulfur (S^{35}) was known to be feasible, permitting tracer experiments.

We incidentally obtained information concerning the effect of various dietary factors on the growth of tapeworms, including sulfasuxidine, various carbohydrates, and thiamin. Information on the source of thiamin for the worms was obtained by fluorimetric analysis of thiamin in worms, intestinal mucosa, and feces under various dietary regimes, and by determination of radioactivity in thiamin extracted from worms and intestinal mucosa, respectively, after parenteral administration of radioactive thiamin. At the conclusion of the paper we have discussed the mechanism whereby tapeworms are able to obtain substances from their hosts, independent of the diet.

MATERIALS AND METHODS

The strain of *Hymenolepis diminuta* employed is the one used in previous experiments reported from this laboratory (Chandler, 1939), maintained in white rats as the definitive host and in *Tenebrio molitor* as the intermediate host. Only adult male rats were used. Worms were removed at autopsy by washing them out of the small intestine with water forced through by means of a large syringe, thus obtaining them unbroken.

It is impracticable to measure worms accurately when freshly removed from the intestine, due to contractions. The worms can be relaxed and contractile movements stopped by placing them in water in a petri dish and leaving them in a refrigerator for 30 minutes or more. They absorb large amounts of water and become paralyzed.

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A preliminary experiment showed that the worms approximately maintain their original weight in normal Tyrode solution. Table 1 shows that all the change, at least in weight *gains*, is complete in the first 45 minutes.

TABLE 1

Strength of Tyrode Solution	Weight change in 45 minutes	Weight changes in 90 minutes
0.6	+23.5%	+22.5%
0.8	+11.0%	+10.5%
1.0	+2.0%	−1.5%
1.2	−7.0%	−11.0%

Beck (1950) showed that the dry weight of *H. diminuta* is 14.4 to 18.7% (av. 16.4%) of the total weight of freshly removed worms. We found the dry weight of worms that had been soaked in water for 60 minutes to be 10 to 11.3% (av. 10.8%) of the wet weight. Assuming that loss of non-aqueous constituents during soaking was negligible, and that the dry weight was therefore approximately the same before and after soaking, it follows that the gain in weight from absorption of water was about 50% of the weight of the unsoaked worms.

In order to get uniform measurements of length the water-soaked worms were laid out on a large glass plate with the aid of a small brush. Maximum width was measured by cutting off the terminal 4 or 5 cm. of the worms, placing these pieces on a microscope slide and measuring them with a micrometer under low magnification. It is obvious that the measurements of the worms obtained by this method are considerably greater than the sizes of the worms as they exist in the intestine of the host, but the measurements are satisfactory for comparative purposes.

Worms and other materials (intestinal mucosa and feces) to be tested for thiamin or radioactivity were placed in special tubes, frozen in acetone and dry ice, and lyophilized. Intestinal mucosa was obtained by slitting open the small intestine, removing adhering fat, and scraping off the mucous membrane with a razor blade.

Methods applicable only to particular experiments are described in connection with those experiments, for the convenience of the reader.

Analysis of the vitamin-deficient diets used in earlier experiments (Chandler, 1943; Addis and Chandler, 1944, 1946) indicated that although undoubtedly deficient from the point of view of the host's nutrition, they had sufficient traces of vitamins so that they may have supplied at least part of the requirements of the tapeworms. In order to eliminate extraneous thiamin as much as possible, a new diet was prepared using the following basic ingredients per rat per day:

sucrose or dextrose	575 gm.
casein	175 gm.
cellulose	88 gm.
corn oil, containing 10 gm. wheat germ oil, and 60 mg. codliver oil	57 gm.
No. 2 salt mixture	41 gm.
SO_2-treated yeast	64 gm.

Each day the requisite amount of the dry materials had the oil added to it and was then moistened with enough water to make a thick paste. The rats were given all they would consume, which averaged about 20 gm. per rat per day. In some

preliminary experiments we used synthetic B-complex vitamins instead of the treated yeast, but changed to the latter because Beck (1950) showed that worms in male as well as in female rats are affected by the absence of an unknown substance or substances in brewer's yeast, although the effect is slower in appearing than in females.

Starch was omitted from this diet because of its vitamin content. At first sucrose was the carbohydrate used, since Guerrant, Dutcher, and Brown (1937) showed that this sugar minimized vitamin synthesis in the intestine. No thiamin was demonstrable in the sucrose by fluorimetric assay. When experiments, described below, showed that sucrose was a less favorable carbohydrate for tapeworm nutrition than dextrose, the latter was used instead of sucrose. The vitamin-test casein used was found on analysis to contain about 1 to 1.5 micrograms of thiamin per gram, so this was further purified by treatment with sodium bisulphite and alcohol, as described by Kline, Hall and Morgan (1941). Our casein was found to contain no demonstrable thiamin after treatment with 2% $NaHSO_4$. The brewer's yeast was freed of thiamin by exposing it in thin layers to an atmosphere of SO_2, with frequent shaking. Analysis showed that from an original 168 μg. of thiamin per gm. dry weight, the thiamin content per gm. was reduced to 4.4 μg. in 2 days, to 2.8 in 3 days, to 1.6 in 5 days, and to 1 μg. or less in 7 days. We therefore treated the yeast for 7 days or longer. Our diet containing sulfite-treated casein and SO_2-treated yeast contained only about 0.1 μg. of thiamin, or less, per rat per day.

In order to minimize coprophagy, cages were used which had bottoms of hardware cloth of $\frac{1}{2}''$ mesh raised well above the dropping pans so no access to the feces was possible after passage. As a final precaution, in some experiments 1% of sulfasuxidine was added to the diet to partially inhibit bacterial synthesis of vitamins in the intestine. Evidence of its effectiveness in this regard has been obtained by a number of authors. In a preliminary experiment involving 12 rats we found, as did Wright and Skeggs (1946), that sulfasuxidine had no harmful effect on the health or growth of rats, and that it had no effect on the establishment or growth of *H. diminuta.*

EFFECTS OF VARIOUS CARBOHYDRATES

The worms obtained from the rats used in the preliminary sulfasuxidine experiment, in which sucrose was the carbohydrate employed, did not attain a length or a width that would be expected of worms in rats on a satisfactory diet, considering their number and age. It occurred to us that this might be due to the substitution of sucrose for dextrose and starch as a source of carbohydrate.

To test this, an experiment was run in which 10 rats, 4 with already mature worms and 6 newly infected with 10 cysticercoids, were placed on a basal diet with untreated yeast and with sucrose as the sole carbohydrate. A similar number of rats were given the same diet except for the substitution of dextrose for sucrose. One rat in the sucrose group and one in the dextrose group harbored no worms at autopsy and are omitted from consideration. The remaining nine rats in each group were considered together since the results in the previously-infected and newly-infected rats (marked * in Table 2) in each group were so similar both in number and size of worms that there was no point in considering them separately. The rats were left on their respective diets for 16 days, when they were autopsied.

Table 2 shows that the worms in rats on a sucrose diet were on the average shorter, narrower, and weighed less than those in rats on a dextrose diet. Statistical analysis was made of the weights in the two groups by taking the average weight of worms for each rat, multiplying each average by the number of worms involved and comparing the totals. The totals were found to be significantly different. If the totals of the averages for each rat were considered without reference to the number of worms in each, the difference was significant if the single abnormally large worm in rat 1 of the dextrose group was rejected.

The conclusion seems justified that sucrose is not as favorable as dextrose as a source of carbohydrate for *H. diminuta*. This may be due to inability of the worms to use sucrose until it is split into dextrose, and the fact that sucrose is largely hydrolyzed in the mucosa rather than in the lumen of the intestine (Florey et al, 1941). This would quite possibly lead to much of the dextrose derived from sucrose being absorbed by the host before the worms had access to it. There is no evidence that the worms have a sucrase of their own.

TABLE 2.—*Comparison of Worms with Sucrose and Dextrose as Sources of Carbohydrate*

Sucrose					Dextrose				
Rat No.	No. Worms	Av. length worms	Av. width worms	Av. wet wt. worms	Rat No.	No. Worms	Av. length worms	Av. width worms	Av. wet wt. worms
1*	10	282.5 mm.	1.99 mm.	0.1364 gms.	1*	1	880.0	3.35	1.3050
2*	4	364.2	1.81	0.1775	2*	9	405.0	3.00	0.4189
3*	6	303.7	2.62	0.1945	3*	2	272.5	2.52	0.2303
4*	2	392.5	2.13	0.2300	4*	8	289.0	2.45	0.1835
5*	1	290.0	1.95	0.1420	5*	4	566.2	3.08	0.6624
6	1	240.0	1.85	0.1247	6*	10	308.5	1.86	0.1648
7	10	310.9	2.15	0.1541	7	10	386.1	2.64	0.3045
8	8	216.5	1.95	0.1069	8	7	374.7	2.91	0.2891
9	8	413.0	2.60	0.2511	9	2	298.0	2.38	0.1840
Av. per worm		311.5	2.17	0.1675 ±.0079			356.8	2.58	0.3158 ±.0307
Av. per rat	5.5	312.7	2.12	0.1686		5.9	373.8	2.69	0.3037

Asterisks indicate new infections.

Although the worms in the dextrose-fed rats did better than those in the sucrose-fed rats, in neither group did the worms grow as long, nor were as many able to survive, as when the rats were fed on a regular pellet diet or on a "synthetic" diet containing starch. To test this, we placed 10 rats given 10-cysticercoid infections on a diet similar to that given the rats in the above experiment, except that the carbohydrate consisted of equal parts by weight of dextrose and starch. The results were striking. The average number of worms per rat was 8.7, with a full quota of ten worms in five of the rats, 9 in one, 8 in two, and 6 in two. The average length of all the worms was 824 mm., the mean in individual rats ranging from an average of 630 to 963, and the average maximum width was 2.71 mm., with the mean in individual rats ranging from 2.39 to 2.93 mm. By comparison with the figures in Table 2 it will be seen that although the average maximum width of these worms was almost exactly the same as in the "dextrose" rats, the mean length was very much greater (824 as compared with 357 mm.). Moreover, there was a marked improvement in number of worms established (8.7 as compared with 5.9).

Since in the rats of the previous experiments there were frequently a few worms much smaller than the majority, we wondered whether the size of the cysticercoids might influence growth, since there is considerable variation when the cysticercoids

are harvested at the end of 18 to 20 days in *Tenebrio*. In the experiment described above, therefore, 6 of the 10 rats were given carefully selected large cysticercoids, whereas the other 4 were given undersized, although apparently mature ones. In the first group of 6 rats, 4 had 10 worms each, 1 had 9, and the other 8. Of the second group of 4, 1 had 10, 1 had 8, and 2 had 6. It would appear, therefore, that a few of the undersized cysticercoids failed to survive. However, there was no evidence of any appreciable diminution in size of the worms that did develop from the small cysticercoids, the average in the large- and small-cysticercoid groups being 832 and 801 mm. respectively. There was no indication that the smaller number of worms (6) in 2 of the rats in the small-cysticercoid group contributed to increased size, for one of these rats had the shortest and the other the longest mean length of worms (630 and 963 mm.) in the entire group.

We have not investigated the reason for the survival and greater length of worms given starch and dextrose as compared with those given dextrose alone, but it seems highly probable that the explanation lies in the fact that dextrose is rapidly absorbed by the host and may not be available in adequate amount for worms or parts of worms situated in posterior portions of the small intestine, whereas the presence of amylase in the intestinal lumen would be expected to make dextrose available to the worms throughout the greater part, perhaps the entire length, of the small intestine when starch is fed. This might also affect the intestinal flora, and thus indirectly affect the worms in some unknown manner.

EFFECT OF THIAMIN-FREE DIET

It has previously been demonstrated by one of us (Chandler, 1943) that thiamin (vitamin B_1) in the host's diet was not necessary for normal growth of *H. diminuta,* and evidence was adduced by Addis and Chandler (1944) that absence of thiamin in the diet actually led to an increase in the size of the worms, presumably by weakening peristalsis and thus permitting more time for evagination of the cysticercoids and establishment in a more favorable, anterior position in the intestine. Since, however, the diets used in these experiments were not as completely free of thiamin as they should have been, an experiment was performed in which 12 rats were placed on our basal diet with 1% sulfasuxidine, and dextrose as a source of carbohydrate. Six of the rats (Group I) had 20 micrograms per day of thiamin added to the diet; the other 6 were left on the thiamin-free diet.

The rats were infected with 10 cysticercoids each on the same day they were put on their diets, thus leaving no time for depletion of thiamin in the tissues of the host. Autopsies were made on the 19th day after infection to allow the worms time for maximum growth.

There was a slightly smaller number of worms in the rats given no thiamin (average 7) than in those that received it (average 8.8), but there was no ill effect on the growth of the worms that succeeded in establishing themselves. In the group without thiamin the average length of the worms was 607 mm. and the average width 2.42 mm.; in the group given thiamin the respective figures were 537 and 2.34 mm. As in Addis and Chandler's (1944) experiments the worms in the thiamin-deficient group are actually larger on the average than those given thiamin, though the number of rats in the experiment was not sufficient to make the difference statistically significant.

THIAMIN ASSAYS OF WORMS, MUCOSA AND FECES UNDER VARIOUS DIETARY REGIMES

Thiamin assays were made by the fluorimetric method described by Hennessy (1947), using a Beckman spectrophotometer, on lyophilized material from rats on various dietary regimes, with and without parenteral thiamin injections. The purpose was to ascertain what fluctuations would occur in the thiamin content of the mucosa under various conditions, and compare this with the amount present in the feces of the rats and in the tapeworms, if any, harbored by them.

Since the spectrophotometer we used had to undergo adjustments several times, increasing or decreasing its sensitiveness, new standards had to be prepared, and the percentage recovery from the eluting column varied from day to day, the results obtained from runs on different days may not always have been comparable, but duplicate runs made on similarly treated animals give confidence in the comparability of runs made on the same day. Results obtained from feces and tapeworms, under comparable conditions, did not vary greatly in any of the runs, but the results from mucosal scrapings were strikingly dissimilar on different days. Since comparable results were obtained from runs on certain dates, 6/24, 7/15, and 10/25, these are pooled in Tables 3, 4, and 5, but runs made on 12/6 and 1/26 are shown separately.

TABLE 3.—*Thiamin Assays on Intestinal Mucosa; µg. per Gram Dry Wt.*

6/24, 7/15 and 1/25	1. 18 days on diet without thiamin; 4 runs on different sets of rats—1.66, 1.74, 1.52, 1.89	Av. 1.70
	2. 18 days on diet with 10% untreated yeast; 3 runs on different sets of rats—14.4, 11.6, 9.7	Av. 11.9
	3. 17 days on diet without thiamin, 10 µg. thiamin injected daily last 8 days; 2 runs on different sets of rat—2.11, 2.61	Av. 2.36
	4. 17 days on diet without thiamin, 50 µg. thiamin injected daily last 8 days; 2 runs on different sets of rats—4.23, 4.00	Av. 4.13
12/6	5. 14 days on diet without thiamin; 4 runs on different sets of rats—4.61, 3.81, 3.07, 3.18	Av. 3.67
	6. 14 days on diet without thiamin, 50 µg. thiamin injected daily last 8 days; 3 runs on different rats—9.60, 10.30, 6.45	Av. 8.78
1/6	7. 14 days on diet without thiamin; 2 runs on different sets of rats—5.14, 4.74	Av. 4.94
	8. 14 days on diet without thiamin, 150 µg. thiamin injected daily 7 days, and also 20 µg. thiamin by mouth in 2 runs; 3 runs on different sets of rats—7.61, 5.83, 6.08	Av. 6.51

Of the various duplicate groups of rats shown in Tables 3 and 4, some had sulfasuxidine added to the diet, others did not; some were given synthetic vitamins except B_1, others SO_2-treated yeast; some were not infected with tapeworms, others were stock rats with old infections, and still others were given fresh 10-cysticercoid infections. Since none of these factors showed any effect on the thiamin content of either the mucosa or the feces, they are not separately shown in the tables. However, assays on the feces of thiaminless rats with and without sulfasuxidine are shown in Table 4, since it might have been anticipated, contrary to the results obtained, that the omission of the sulfa drug would have influenced the amount of fecal thiamin.

Examination of Table 3 shows that on four runs made on 6/24, 7/15 and 10/25, on different sets of rats given no thiamin either by mouth or parenterally (No. 1 in the Table), the amount of thiamin detected in the intestinal mucosa was in each instance less than 2 µg. per gram dry weight, averaging 1.7. Rats injected with 10 µg. of thiamin daily for 8 days averaged 2.36 µg. per gram and those given 50 µg. daily for 8 days averaged 4.3 µg. per gram. In the runs made on 12/6 and 1/6, however, the thiamin assays averaged 3.67 and 4.94 µg. These rats were kept thiamin-free

for only 14 days instead of 17, but it does not seem likely that this accounts for the difference. The relative difference between the thiamin assays on mucosal scrapings from rats given no thiamin and those given parenteral injections of 50 μg. daily is about the same in the runs made on 12/6 as they were in the earlier runs. The high results obtained on 1/6 on rats given no thiamin have not been satisfactorily accounted for. The very high figures obtained for the mucosa in rats given untreated brewer's yeast (No. 2 in Table 3) were probably due to failure to wash all of the yeast out of the mucosa before the intestines were scraped.

From the data given in Table 3, the conclusion seems warranted that the amount of thiamin in the mucosa is reduced by 14 to 17 days without thiamin by mouth or injection, and is definitely increased by parenteral injections.

Results of thiamin assays of the feces are shown in Table 4. It will be seen that in all the groups (Nos. 1, 2, 4, and 5) in which thiamin was eliminated from the diet, even when the excessive dose of 50 μg. per day was injected parenterally each day for 8 days, the thiamin in the feces ran consistently low, ranging from 1.6 to 2.3 μg. per gram dry weight, and averaging 1.8 to 2 μg. When 20 μg. per day of thiamin was given by mouth (No. 3) not all of it was absorbed, for the amount of thiamin

TABLE 4.—*Thiamin Assays on Feces; μg. per Gram Dry Wt.*

6/24, 7/15 and 10/25	1. 17 or 18 days on diet without thiamin, with 1% sulfasuxidine; 7 runs on different sets of rats—1.93, 1.60, 1.65, 2.36, 2.22, 1.91, 2.35	Av. 2.00
	2. 14 days on basal diet without thiamin, and with no sulfasuxidine	Av. 1.86
	3. 18 days on basal diet + sulfasuxidine, with 20 μg. thiamin added to food daily	Av. 3.97
	4. 17 days on diet without thiamin, + sulfasuxidine, but 10 μg. thiamin injected daily last 8 days; 2 runs on different sets of rats —1.91, 1.90	Av. 1.91
	5. 14–17 days on diet without thiamin, + sulfasuxidine, but 50 μg. thiamin injected daily last 8 days—1.86, 1.69, 1.91, 1.81	Av. 1.82
	6. 18 days on basal diet with 10% untreated yeast instead of the usual amount of SO_2-treated yeast; 2 runs on different sets of rats—66.8, 81.8	Av. 74.30

excreted in the feces was significantly increased. The large amounts of thiamin found in yeast-fed animals (No. 6) is evidence that considerable amounts of the thiamin in the yeast was not taken up by the animals.

From the evidence afforded by Table 4 it may be concluded that about 2 μg. of thiamin per gram of dry weight of feces is close to an irreducible minimum, even when sulfasuxidine is added to the food to reduce bacterial synthesis. Probably most of this thiamin is produced by yeasts in the cecum (Guerrant, Dutcher, and Tomey, 1935). These authors found evidence of very little synthesis in the small intestine. As various authors have pointed out, e.g., Leong (1937), Alexander and Landwehr (1945), there is evidence that most of the fecal thiamin in hosts on diets not containing a large excess of thiamin is retained in cells of microorganisms and is not available to either hosts or parasites.

The fact that the amount of thiamin in the feces is not increased by parenteral injection of excessive amounts of thiamin into the host is evidence that thiamin is not lost from the intestine via the feces, although it probably diffuses into the intestine and is reabsorbed (Read, 1950a). Similar conclusions were reached by Emerson and Obermeyer (1945), who found the same concentration of thiamin in the feces of thiamin-starved animals as in animals fed 5 or 50 μg. of thiamin daily. Wildemann (1941) got similar results even with 250 μg. daily, and Denko et al (1946)

reported fecal excretion of vitamins to be unaffected by vitamin supplementation of diets.

The large amounts of thiamin found in the feces of rats given 10% of untreated yeast in the diet is not unexpected, since Leong (1937) showed that when the intake of thiamin is large, appreciable amounts escape absorption and are excreted in the feces.

We conclude from these experiments of our own and others that microorganisms do not make appreciable amounts of thiamin, and possibly not of most other vitamins, available to tapeworms residing in the small intestine, and that there is no appreciable loss of parenterally introduced thiamin via the feces, a fact which is of importance in connection with the experiments with injected radioactive thiamin described below.

Results of thiamin assays of worms are shown in Table 5. It will be seen that with the exception of one lot of tapeworms soaked in water and measured before being lyophilized, the amount of thiamin found in the worms was quite consistent, averaging between about 4 and 5 μg. per gram dry weight whether or not thiamin was

TABLE 5.—*Thiamin Assays of Tapeworms; µg. per Gram Dry Wt.*

1. Old worms, hosts on stock pellet diet, placed in lyophilizing tubes and frozen at once—4.18, 5.21	Av. 4.70
2. Same, but worms soaked in water 30 minutes and measured before lyophilizing	Av. 2.80
3. From hosts 14 to 18 days on thiamin-free diet—5.36, 2.95, 5.21, 4.14	Av. 4.41
4. From hosts 14 days on thiamin-free diet, given 150 µg. radioactive thiamin injections daily last 7 days—4.57, 5.11	Av. 4.84
5. From hosts 14 days on basic diet plus 20 µg. normal thiamin daily, given 150 µg. radioactive thiamin injections last 7 days	Av. 5.37

present in the diet, or was parenterally injected. Since the figure obtained for the soaked worms was markedly lower (2.8 μg.) than in unsoaked worms lyophilized at once (4.1 and 5.2 μg.), all other thiamin determinations were made on worms washed out of the intestine with Ringer's solution, rinsed, and immediately frozen in the lyophilizing tubes. In one subsequent run a low figure of 2.95 μg. was obtained, so the difference between soaked and unsoaked worms may not have been significant. The thiamin content of *Hymenolepis diminuta,* as determined by the fluorimetric method, is somewhat lower than that of *Moniezia benedeni* (8.6 μg. per gram) as determined by a biological assay by Chance and Dirnhuber (1949).

EXPERIMENTS WITH RADIOACTIVE THIAMIN

In view of the facts demonstrated by our thiamin assays of mucosa, feces, and worms, we felt that by injecting thiamin labeled with radioactive sulfur (S^{35}) into thiamin-starved hosts and comparing the specific activity of the thiamin in the worms with that in the intestinal mucosa, we might be able to determine with certainty whether the tapeworms acquired their thiamin directly from the host or from some other source.

Borsook et al (1940) reported some experiments on the course of thiamin metabolism in man as indicated by radioactive sulfur. The method of synthesis of the radioactive thiamin by them, as well as by us, was essentially that of Cline, Williams and Finkelstein (1937), which involved the condensation of 2-methyl-5-bromoethyl-6-aminopyrimidine hydrobromide with 4-methyl-5-beta-hydroxyethylthiazole, followed by conversion of the resulting thiamin bromide-hydrobromide into the cor-

responding chloride-hydrochloride. In the synthesis we were concerned only with the preparation of the thiazole fraction, which contained the S^{35}. The necessary pyrimidine fraction (in the form of the 5-ethoxymethyl precursor) was very kindly supplied to us gratis by Merck and Co., to whom we express our sincere thanks.

Since the synthesis of the thiazole fraction presented some difficulties, we believe it is worth while to give some details of its preparation. In the first attempt we followed the procedure as given by Buchman (1936), in which S^{35} is combined with red phosphorus to give P_4S_{10}. This in turn is allowed to react with formamide to produce the needed thioformamide. The final step is the reaction between thioformamide and 3-aceto-3-chloro-propanol-1 to produce the required thiazole. We are also greatly indebted to Merck and Co. for generously supplying us with a large sample of the above-mentioned aceto-chloro-propanol. The difficulties encountered in attempting to use this method were due to our inability to prepare the P_4S_{10}, since when commercially prepared P_4S_{10} (J. T. Baker Chem. Co.) was used, excellent yields of the thiazole were obtained.

We next attempted a method for the synthesis of the thiazole ring as reported by Hatcher (1947), which involved the condensation of thiourea with the same aceto-chloro-propanol, followed by subsequent removal of an unwanted amino group on the thiazole ring. After several trial runs with ordinary thiourea, fairly good yields of the desired thiazole were obtained by this method. Through the cooperation of the Abbott Laboratories we obtained some thiourea containing S^{35}, with a specific activity of 9 millicuries per gram. With this, one of us (H.O.N.) succeeded in preparing a sample of the radioactive thiazole, which when condensed with the pyrimidine fraction gave us about 150 mg. of a product which proved to contain about 50% isotopically labeled thiamin, established by spectrophotometric analysis for thiamin and by its specific radioactivity. The impurity was believed to be 2-methyl-5-chloromethyl-6-aminopyrimidine hydrochloride which had failed to react with the thiazole. We tested this substance for toxicity to rats, and found no evidence of harmful effects.

An experiment was performed employing 12 rats which had been infected with 10 cysticercoids each 3 to 6 weeks earlier. All were put on our basic diet with SO_2-treated yeast. These rats were divided into 3 groups of 4 rats each. The rats of Group I were given no thiamin either by mouth or by injection; those of Group II were given daily intraperitoneal injections of 150 μg. radioactive thiamin (300 μg. of our impure product) for 7 days, after the first 7 days on the thiamin-free diet; Group III had 20 μg. per day of normal thiamin added to the diet throughout the 14 days of the experiment, and were given daily injections of 150 μg. of radioactive thiamin on the last 7 days.

The results of this experiment are shown in Table 6. It will be observed that both in the rats that received no thiamin by mouth but only radioactive thiamin by injection, and in those that received normal thiamin by mouth as well as radioactive thiamin parenterally, the specific activity of the thiamin extracted from the worms and that extracted from the intestinal mucosa are remarkably similar; so similar, in fact, as to be identical, within the limits of experimental error. Most of this error probably is in the thiamin extraction and determination rather than in the determination of radioactivity. It will be observed that the figures for specific activity of

thiamin are somewhat lower in the animals receiving both normal and radioactive thiamin than in those receiving radioactive thiamin only, as would be expected.

The close similarity in specific activity of the thiamin extracted from the worms and from the intestinal mucosa, in the light of our evidence that even when large doses of thiamin are parenterally administered none is excreted into the intestine and lost in the feces, can only be interpreted to mean that the thiamin in the worms is obtained from the host. If even a moderate fraction of it had been obtained from other sources, e.g., from the diet, from synthesis by microorganisms in the intestine, or from synthesis by the worms themselves, a lower specific activity in the worms would have been expected.

If the worms can obtain thiamin from the hosts' tissues there is good reason to suppose, in the light of the lack of dependence of the tapeworms on the presence of any of the known vitamins in the diet of the host (Chandler, 1943; Addis and Chandler, 1944, 1946) that other vitamins are likewise obtained from this source. It is quite possible that the absorption of various vitamins may differ with different species of tapeworms, and one is led to speculate that the pernicious type of anemia

TABLE 6.—*Specific Activities in Mucosa and Worms After Injection of Radioactive Thiamin*

Group		Thiamin Assays (µg. Thiamin per Gram Dry Wt.)		Specific Activity (Counts per Minute per µg. Thiamin)	
		Mucosa	Worms	Worms	Mucosa
I. No thiamin 14 days	rats 1 and 2	4.74	2.95		
	rats 3 and 4	5.14			
II. No thiamin in diet 14 days; 150 µg. radioactive thiamin daily last 7 days	rats 5 and 6	7.61	5.11	99	91
	rats 7 and 8	no worms			
III. 20 µg. normal thiamin in diet 14 days; 150 µg. radioactive thiamin daily last 7 days	rats 9 and 10	5.83	5.37	62	71
	rats 11 and 12	6.08	4.57	75	90

sometimes produced by *Dibothriocephalus latus* may possibly be due to an unusual affinity of that worm for Vitamin B_{12}, thus precipitating pernicious anemia in individuals in whom suboptimal amounts of the antianemia factor are produced in the liver. On the other hand, members of the "folinic acid complex" (Shive, 1950) may be involved. It may also be, as Chandler (1943) suggested, that tapeworm toxicity in general may largely be an induced vitamin deficiency resulting from absorption of vitamins from the tissues of hosts subsisting on a suboptimal diet.

POSSIBLE MECHANISM BY WHICH TAPEWORMS OBTAIN SUBSTANCES FROM THE HOST

Although the evidence is now quite definite that tapeworms acquire adequate amounts of nitrogenous foods, vitamins, and perhaps other substances from the host, independent of the diet, there is the further question of the means by which this is accomplished. It was intimated by Chandler (1943) that nutriments might be directly absorbed by tapeworms from mucosal cells, but it now appears that no such improbable mechanism is necessary. An extensive survey of the literature on the nature and behavior of the intestinal mucosa and associated glandular structures by one of us (Read, 1950a) has suggested a simpler answer to this question.

Contrary to the usual concept of the intestinal wall as a membrane which, except for its secretory glands, permits one-way passage of substances from the lumen to the interior of the villi, there is now a large mass of evidence to indicate that to a

very surprising extent the cells lining the lumen of the intestine permit two-way passage of a great many substances, the direction of passage being determined in some instances by simple diffusion, and in others by "active transfer" processes in which energy is expended. We shall briefly consider the physiology of the intestine with respect to (1) oxygen, (2) nitrogenous materials, and (3) vitamins.

1. Oxygen. It was demonstrated by Chandler (1939) that the size and rate of growth of *Hymenolepis diminuta* was in inverse ratio to the number of worms present, and also that an existing infection had an inhibitory effect on the development of worms of a superimposed infection. The latter phenomenon was shown to be due not to development of a specific immunity, but to a crowding effect, resulting in disadvantageous competition for some substance or substances needed in the metabolism of the worms. A single worm in the lumen of the intestine would have almost its entire surface in contact with the walls of the intestine from which it could obtain substances necessary for its growth, whereas, as the worms increased in number, there would be more and more contact of worms with one another and less and less with the mucosa. Furthermore, worms of a secondary infection would be at a disadvantage in this respect from the very beginning of their period of growth.

Upon finding that nitrogenous foods and vitamins could be acquired from the host independent of its food, it was thought that competition for these substances alone might account for the phenomena observed. However, it now seems possible that an even more critical substance needed by the worms in their metabolism, and restricted by crowding, is oxygen. The relation of the crowding in tapeworm infections to the area per unit of weight of the individual worm and the interpretation of this relation in terms of the oxygen requirement has been discussed elsewhere (Read, 1950b).

2. Nitrogenous materials. Study of the literature indicates that the quantity of nitrogen-containing substances passing into the intestine from the body of the host is surprisingly large, amounting to about 35% of the quantity ingested by an animal on a normal diet. The variety of these substances that enter the gut from the hosts' body is amazing. In the succus entericus have been identified protein, amino acids, urea, creatine, creatinine, and ammonia. The bile may contain all of these and also "mucin," uric acid, and allantoin. The pancreatic secretion contains significant quantities of nitrogen-containing materials, but the exact nature of these substances has been little investigated. A variety of nitrogenous compounds, including amino acids, are normal constituents of the gastric juice which passes into the small gut.

3. Vitamins. A number of vitamins are known to enter the intestinal lumen from the parenteral tissues. Vitamin D occurs in the succus entericus and in the bile. Ergosterol has been isolated from the bile of the ox and the dog. Ascorbic acid, riboflavin, and probably B-12 are present in the succus. Vitamin "B" has been reported as a bile constituent. Inositol is present in the pancreatic juice. The presence or absence of vitamins in the intestinal secretions has not been comprehensively investigated, but the data available indicate that such investigations would be very illuminating. In the present communication we have presented strong circumstantial evidence that thiamin passes into the gut from the host tissues, and also that it is re-absorbed.

Still other materials from the host tissues are available to tapeworms. Moderate amounts of fatty materials are present in the intestinal secretions, though there is

no evidence that tapeworms require them. In addition, certain hormones or hormone-like substances, including estrogens and androgens, are present in the host secretions. Evidence of need for these by tapeworms has been adduced by Addis and Chandler (1946) and by Beck (1950).

These aspects of intestinal physiology serve to emphasize the conclusion reached by Read (1950a) that the gut is in a dynamic relation with the host tissues and that full understanding of the host-parasite relationship can be attained only by *separately* studying host and parasite physiology. Merging or re-synthesis of the separate knowledge of the host and parasite may provide entirely new concepts of the host-parasite relation.

SUMMARY

To study the source of thiamin for *Hymenolepis diminuta,* a thiamin-free diet was prepared including vitamin-test casein retreated with sulfite, SO_2-treated yeast, 1% sulfasuxidine (found to be harmless to the worms), and sucrose or dextrose as the sole carbohydrate. Sucrose is less favorable as a source of carbohydrate than dextrose, and neither is as favorable as a mixture of starch and dextrose. Practically complete elimination of thiamin has no adverse effect on growth of the tapeworms.

The thiamin content of the intestinal mucosa is reduced by a thiamin-free diet and is increased by parenteral injections of thiamin. Thiamin in the feces, on the other hand, is not increased even by excessive amounts of parenterally injected thiamin. However, even after prolonged elimination of thiamin from the diet, the feces contain a fairly constant small amount, probably contained mostly in the bodies of microorganisms and not available to either hosts or parasites. The thiamin content of *H. diminuta* remains fairly constant regardless of its presence or absence in the diet, or of parenteral injections of it.

After parenteral injection of radioactive thiamin, the thiamin in the tapeworms and in the intestinal mucosa of the hosts have the same specific activity, indicating that the thiamin of the worms was obtained from the host.

The intestinal lumen not only receives a great variety of substances by secretion of its own and associated glands, but also many substances by diffusion. Parasites in the lumen thus have access to nitrogenous substances, vitamins, hormones, oxygen, etc., and are therefore at least partly independent of these materials in the host's food. Oxygen secreted into the lumen may be the critical substance, needed by the worms, which accounts for the "crowding effect."

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STUDIES ON BOVINE GASTRO-INTESTINAL PARASITES XVI. SOME RESULTS OF FEEDING SMALL AMOUNTS OF PHENOTHIAZINE ON PURE INFECTIONS OF THE HOOKWORM *BUNOSTOMUM PHLEBOTOMUM*

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As a result of life history studies of three of the important parasites of calves in which it was found that the symptoms of parasitism develop during the larval period rather than during the adult life of the worms (Mayhew 1944, 1948a, 1948b, 1949, and Delaune and Mayhew 1941, 1943), we concluded that prevention of infection was exceedingly important in controlling losses. This end may be gained either by pasture rotation when infection takes place by way of the mouth, by barn and shade sanitation in the case of the skin penetrating species, and by the destruction of the larvae before infection can occur. The latter objective might be accomplished by mixing some lethal chemical with the manure of the infected animal or by feeding a chemical that would pass through the digestive tract of the infected animal in sufficient quantity to destroy the developing larvae. The experiments of Shorb and Habermann (1940) and Habermann and Shorb (1942) in which they failed to recover infective larvae after feeding small amounts of phenothiazine to sheep, suggested the beneficial possibilities of the use of this drug in calves. In the following pages are described the results of some experiments in which small amounts of the drug were given with the daily grain ration fed to animals harboring pure infections of the hookworm *Bunostomum phlebotomum.*

METHODS

The calves used in these experiments were obtained when a few hours old and raised parasite free except for *Strongloides* sp. until they were inoculated with pure cultures of infective larvae of the cattle hookworm. They were first used in life history studies of this parasite and as these data were completed they became available for the phenothiazine experiments. The methods of caring for the animals were the same as used in the other experiments reported in this series of papers and need not be redescribed at this point. The weighed amounts of phenothiazine were mixed with the regular amount of the commercial grain ration and fed in the late afternoon.

EXPERIMENTAL RESULTS

No. 159. This animal was a pure bred Holstein male born at the L.S.U. Dairy Department on October 11, 1945. He was inoculated with hookworm larvae on May 26, 1946. The resulting infection described in a previous paper (Mayhew 1948) proved to be well established and as a consequence the animal was used as a source of larval cultures for other inoculations until October 1947. Fecal examinations were made at regular weekly intervals until October 15, 1947 when daily examinations were begun. Typical hookworm eggs only were observed in a total of 88 positive examinations made previous to October 15th.

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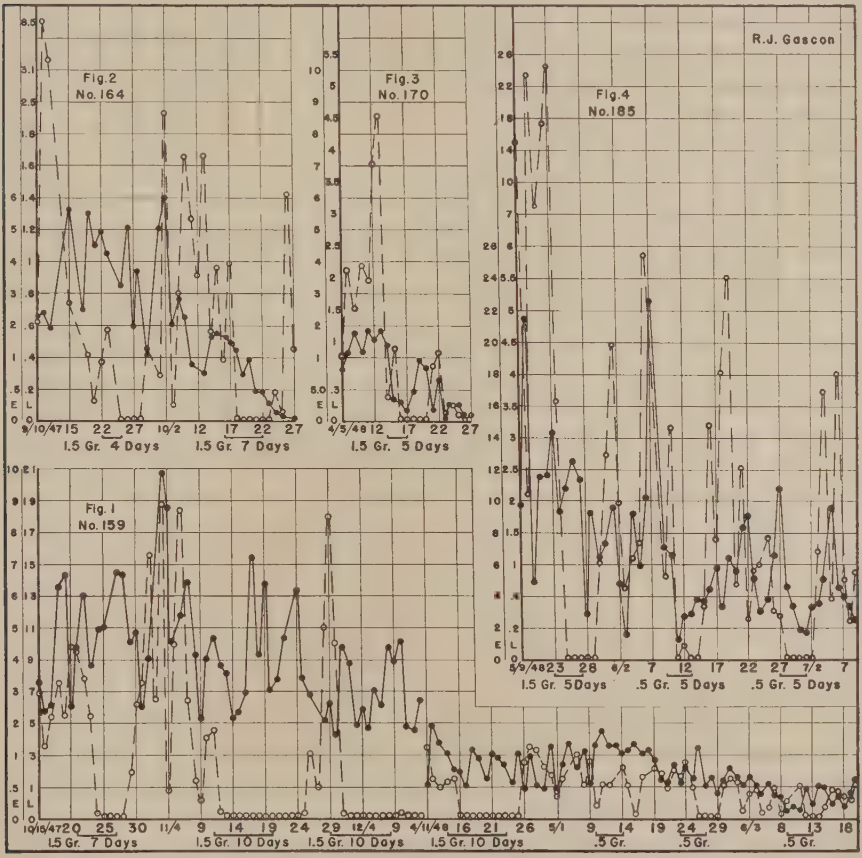
The first phenothiazine feeding experiment was begun on October 21, 1947 at which time the calf was approximately 2 years of age. One and one-half ($1\frac{1}{2}$) grams of the drug was fed daily for 7 days. The results (fig. 1) indicate that egg production was not interfered with, but that infective larvae were not recovered in daily cultures between October 24th, the 3rd day after the first feeding, and October 29, the second day after the last feeding of the drug. Daily fecal examinations and larval cultures were continued and, it was found that egg production continued and infective larvae were recovered daily following their reappearance (fig. 1).



Explanation of Figs. 1 to 4. Graph showing egg counts (solid lines) and larvae counts per gram of manure.

On November 11th a second feeding of phenothiazine was begun and it will be noted, (fig. 1), that infective larvae were not recovered from November 13th, the second day after the first feeding until November 25th, the fifth day after the last feeding of phenothiazine. In this experiment $1\frac{1}{2}$ grams of the drug were fed daily for a period of 10 days.

Daily cultures and fecal examinations were continued and a third experiment was begun on November 29th in which $1\frac{1}{2}$ grams of phenothiazine were fed daily until

December 8th, a period of 10 days. Infective larvae were consistently recovered until December 1, the second day after the first feeding, and they were absent from the cultures except on December 10th when only a very few were found, until December 16th when cultures were discontinued. It is believed that the failure of larvae to reappear as in the other experiments was due to the cold weather at the time since the cultures were not under controlled temperature conditions.

Larval cultures were not begun again until April 5th, 1948 (fig. 1). Infective larvae were consistently recovered from the daily cultures and daily fecal examinations showed the presence of normal eggs. Another series of 10 daily feedings of $1\frac{1}{2}$ grams of phenothiazine was begun on April 14th and continued through the 23rd. On April 16th, the second day after the first feeding, (fig. 1) infective larvae disappeared from the cultures and did not reappear again until April 26th, the third day after the last feeding of the drug.

Daily larval cultures were continued, with the exception of May 6th, and it will be noted, fig. 1, that larvae were consistently recovered from all. We now decided to try some experiments in which a smaller amount of the drug is used. One half gram of phenothiazine was fed for a period of 5 days beginning May 10, 1948. It will be noted (fig. 1) that infective larvae were recovered daily during the time when they were absent in former experiments. A second experiment in which $\frac{1}{2}$ gram was fed for 5 days was conducted between May 23rd and 27th with the result that larvae were not recovered for 4 days beginning on May 26th, the third day after the first feeding. A third experiment, using $\frac{1}{2}$ gram for 5 days, was carried out between June 9th and 13th with the result that infective larvae were not recovered from the 12th, the third day after the first feeding, to the 14th. Larvae were consistently present in daily cultures from June 15th to the 24th. Further experiments were not carried out with this animal and he was destroyed on August 20, 1948 and seven adult female hookworms were recovered. The fact that larvae were recovered during one of the three experiments in which $\frac{1}{2}$ gram was fed, suggests that this amount is approximately the minimum amount that interferes with larval development.

No. 164. This animal was a pure bred Holstein male born at the L.S.U. Dairy Department on November 15, 1946 and was inoculated with hookworm larvae on December 16, 1946 and on May 9, 15 and 19, 1947. The results of these inoculations were reported in a former paper (Mayhew 1949). He weighed 320 pounds on September 12, 1947 at the age of 10 months. A series of 4 daily feedings of $1\frac{1}{2}$ grams of phenothiazine was begun on September 22, 1947. The results (fig. 2) show that there was no interference with egg production, but that no infective larvae were recovered from September 25th, the third day after the first feeding, to the 28th, the third day after the last feeding of the drug. After September 28th normal larvae were recovered consistently from the cultures. A second experiment in which $1\frac{1}{2}$ grams was fed for 7 days was begun on October 16th. We again failed to recover infective larvae from the third day after the first feeding, October 19th, through the first day after the last feeding, October 23rd. Larvae were regularly recovered from October 24th on. The egg counts were so low following this experiment that the animal was discarded. A total of 37 larvae cultures were made between September 8th and October 27th, the days on which no cultures were made were September 9, 12, 13, 14, 16, 17, 20, 21, 24 and 30.

No. 170. This calf was a pure bred Holstein male born at the L.S.U. Dairy Department on May 21, 1947. He was inoculated on May 23rd, 1947 the results of which have been described in Mayhew 1949. A series of 5 daily feedings of 1½ grams of phenothiazine was begun on April 14, 1948. It will be noted (fig. 3) that egg production was not affected but that infective larvae were not recovered from cultures from April 16th, the 2nd day after the first feeding, to the 20th, the second day following the last feeding. Larvae were consistently recovered from daily cultures from April 5th to 25th except from the 16th to the 20th as stated above. He was approximately 11 months old at the time of the experiment.

No. 185. This animal was a pure bred Jersey male born at the L.S.U. Dairy Department on November 24, 1947. He was inoculated with hookworm larvae on February 4, 1948 and the results are described in Mayhew 1949. The results of the 3 phenothiazine feeding experiments carried out with this animal are shown graphically in figure 4. In the first, 1½ grams of phenothiazine was fed for 5 days from May 23rd to 27th. It will be noted that egg production was not affected but that infective larvae were not recovered from May 25th, the second day after the first feeding, to May 29th, the second day after the last day on which the drug was fed.

A second experiment was begun on June 9th in which ½ gram was fed daily for 5 days or until June 13th. Larvae were absent on June 11th, the second day after the first feeding, and on the 13th and 14th, but a few were present on June 12 (fig. 4). In the daily cultures following June 15th, larvae were consistently present. Another experiment in which ½ gram of the drug was fed was started on June 27th and continued through July 1. It will be noted that infective larvae were not recovered between June 28th, the first day following the first feeding and July 2nd. Larvae were consistently recovered from the cultures made during the 8 days following July 3rd. This calf weighed 247 pounds on August 9, 1948 at the age of 8½ months. During the post-mortem examination 3 hookworms were found, two were males and one was a female. The results of these last two experiments on No. 185 in which ½ gram of phenothiazine was fed suggests that possibly ½ gram is the approximate minimum dosage that will interfere with larval production since larvae were recovered on one of the days during the first experiment when we would not have expected to find any.

SUMMARY

The results of eight experiments in which 1½ grams of phenothiazine was fed daily in the grain ration to calves with pure infections of the hookworm indicate that egg production is not affected but that infective larvae fail to develop in the manure. Four different animals were used in these experiments ranging in age from six months to two years.

The results of five experiments using ½ a gram indicate that this is probably the approximate minimum amount that will interfere with larval development, since larvae were recovered during two of the experiments.

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EXPERIMENTAL COPEPOD HOSTS OF THE BROAD TAPEWORM OF MAN, *DIBOTHRIOCEPHALUS LATUS* (L.)

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INTRODUCTION

Janicki and Rosen (1917) first demonstrated that copepods serve as intermediate hosts of the broad tapeworm of man, *Dibothriocephalus latus* (L.).* They reported that the procercoids developed only in *Cyclops strenuus* Fischer and *Diaptomus gracilis* Sars, although coracidia were ingested by other copepods, namely, *Cyclops viridis* (Jurine), *C. oithonoides* Sars, *C. macrurus* Sars, *C. vernalis* Fischer, and probably also *C. leuckarti* Claus.

Diaptomus graciloides Lilljeborg was easily infected by Redlich (1925). This investigator confirmed the development of procercoids in *Cyclops strenuus* Fischer, but obtained negative results with *C. albidus* (Jurine), *C. viridis* (Jurine), and *C. serrulatus* Fischer.

Essex (1927) succeeded in infecting a few *Cyclops brevispinosus* Herrick (4 out of 110) and *C. prasinus* Fischer (3 out of 24). His data suggested that these species were not natural hosts. He was unable to infect *Cyclops bicuspidatus* Claus (132 specimens), *C. leuckarti* Claus (91), *C. serrulatus* Fischer (13), *C. ater* Herrick (3), *C. fimbriatus* Fischer (3), and *C. albidus* (Jurine) (2). Essex found, however, that in *Diaptomus oregonensis* Lilljeborg procercoids developed in a way similar to that described by Janicki and Rosen for *Cyclops strenuus* Fischer. Although Essex credited Janicki and Rosen with reporting *Diaptomus castor* (Jurine) as a first intermediate host, no reference to this species is made in their (1917) paper.

Nicholson (1928), discussing *D. latus* in Manitoba, mentioned *Cyclops strenuus* Fischer as a first intermediate host, evidently referring to the work of Janicki and Rosen, since he gave no original evidence of this copepod serving as either a natural or experimental host. According to Yeatman (1944), there are no authentic records of *C. strenuus* Fischer in North America. Hall (1929), in discussing arthropods as intermediate hosts of helminths, listed *Cyclops robustus* Sars as a host of *D. latus*.

Vogel (1930) discovered that *Diaptomus vulgaris* Schmeil could be regularly infected, and confirmed the observation of Janicki and Rosen that *D. gracilis* Sars was susceptible to infection. Working with two morphologically distinct strains of *Cyclops strenuus* Fischer, he was able to infect a certain percentage of one strain, but the other strain resisted infection entirely. Attempts to infect *Cyclops viridis* (Jurine), *C. albidus* (Jurine), *C. bicuspidatus* Claus, and *C. serrulatus* Fischer were unsuccessful.

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* Although this tapeworm has been commonly known as *Diphyllobothrium latum* (L.), it is the opinion of Wardle, McLeod, and Stewart (1947) that the generic term *Diphyllobothrium* Cobbold should be reserved for certain tapeworms from toothed whales, the correct generic name for the broad tapeworm of man being Lühe's *Dibothriocephalus*.

Magath and Essex (1931) demonstrated that procercoids developed in *Diaptomus sicilis* Forbes and *D. siciloides* Lilljeborg.

Michajlow (1932) found that *Cyclops strenuus* Fischer already infected with procercoids of *Triaenophorus* 28 days old could be infected with larvae of *D. latus*. The procercoids of the latter developed normally and eventually caught up with the procercoids of *Triaenophorus* in development. Evidently there was no immunity to procercoids developed as a result of the first infection.

Michajlow and Wierzbicka (1935) obtained practically 100 per cent infection in large numbers of both sexes of *Diaptomus gracilis* Sars. Other species of copepods gave varying results. Only 2 out of 100 specimens of *Cyclops vicinus* Uljanin showed larvae in the body cavity, and only one harbored a procercoid after 13 days. In *C. strenuus* Fischer 5 out of 45 specimens had larvae in the body cavity, and none of these developed to the cercomer stage. In *C. vicinus* var. *kikuchii* Smirnov 6 out of 50 had larvae in the body cavity, but only one reached the stage of having a cercomer and calcareous bodies. Only 1 out of 8 *C. serrulatus* Fischer showed larvae in the body cavity. Twenty-one specimens of *C. bicuspidatus* Claus remained uninfected.

Vergeer (1936) reported negative results from feeding coracidia to *Cyclops bicuspidatus* Claus (138 specimens), *C. americanus* Marsh (45), *C. brevispinosus* Herrick (14), *C. fimbriatus* Fischer (12), *C. prasinus* Fischer (11), *C. serrulatus* Fischer (11), *C. albidus* (Jurine) (10), *C. leuckarti* Claus (9), *C. ater* Herrick (3), and *C. phaleratus* Koch (1). Coracidia were ingested by 31 *Diaptomus minutus* Lilljeborg, but oncospheres reached the body cavity in only two. In both copepods the oncospheres were reduced to masses of unrecognizable tissue and failed to develop into procercoids.

Michajlow (1938), working with *Triaenophorus lucii* (Müller), proposed five categories of copepod-tapeworm relationships. These were (1) all coracidia are digested by the copepod, (2) most larvae degenerate in the intestine and the few which do enter the body cavity are arrested in growth, developing no cercomers or calcareous bodies, (3) considerable number reach the body cavity but development is hindered, (4) most larvae degenerate in the intestine, but the few which reach the body cavity develop into normal procercoids, and (5) larvae penetrate en masse into the body cavity and develop normally. He considered that groups 1, 2, and 3 were distinct, and that groups 4 and 5 showed intergrading forms. Natural copepod hosts would fall in groups 4 or 5, the principal intermediate hosts being in group 5.

Skvortzov and Talysin (1940) reported *Cyclops strenuus* Fischer from Lake Baikal as a first intermediate host of *Diphyllobothrium minus* Cholodkovsky, a species regarded by some as being identical with *D. latus*.

MATERIALS

Coracidia for experimental infections were obtained from dogs which had been fed plerocercoids of *Dibothriocephalus latus* taken from the muscles of great northern pike (*Esox lucius* L.), Lake Winnipeg, Manitoba. These plerocercoids were made available through the kindness of Dr. M. S. Ferguson of the United States Public Health Service. Eggs recovered from the dog feces were allowed to develop at room temperature (about 21 degrees C.) for about 10–15 days, when hatching began. Copepods were placed for varying lengths of time in fingerbowls containing

both coracidia and eggs. After exposure of several hours the copepods were removed to bowls of fresh pond-water, where they were kept at room temperature. Copepods were examined at intervals until the number was exhausted. Since species of *Diaptomus* were difficult to maintain under laboratory conditions over long periods of time, many of these copepods died before late development of the procercoids could be observed.

EXPERIMENTAL RESULTS

Diaptomus piscinae Forbes.

This species was collected from a small pond near a sewage disposal area at Concord, Massachusetts. Procercoids developed in 55.4 per cent of 92 copepods,

TABLE 1.—*Experimental* D. latus *infections in copepods*

	Total number of copepods	Per cent infected	Average number of procercoids	Number of males included in total	Per cent infected	Average number of procercoids	Number of females included in total	Per cent infected	Average number of procercoids
Diaptomus piscinae Forbes	92	55.4	2.7 (1 to 8)	53	51.0	3.1 (1 to 8)	39	61.0	2.3 (1 to 7)
Diaptomus sanguineus Forbes (including both copepodid stage V and adults)	159	42.1	1.9 (1 to 6)	64	47.0	1.9 (1 to 6)	95	40.0	1.9 (1 to 6)
D. sanguineus F. (copepodid stage V only)	40	55.0	2.3 (1 to 5)	12	66.0	2.1 (1 to 3)	28	50.0	2.4 (1 to 5)
D. sanguineus F. (adults only)	119	38.6	1.6 (1 to 6)	52	42.0	1.6 (1 to 4)	67	36.0	1.5 (1 to 6)
Diaptomus mississippiensis Marsh (infected in Georgia)	89	91.0	3.2 (1 to 10)	30	80.0	3.4 (1 to 8)	59	96.0	3.1 (1 to 10)
Diaptomus mississippiensis Marsh (infected in Boston)	29	27.6	1.2 (1 to 2)	11	18.0	1.0 (1)	18	33.0	1.5 (1 to 2)
Eurytemora affinis Poppe	24	83.3	5.8 (1 to 30)	9	88.0	7.0 (1 to 30)	15	80.0	4.6 (1 to 13)
Diaptomus albuquerquensis Herrick	Susceptible to infection but detailed infection data not available.								

with an average number of procercoids per copepod of 2.7 (1 to 8). The infection rates by sexes are shown in Table 1. Procercoids reached the cercomer stage with calcareous bodies 10 to 13 days after infection. The oldest procercoids were observed 29 days after infection.

Diaptomus sanguineus Forbes.

The source of this species was a small pasture pond at Weston, Massachusetts. Procercoids developed in 42.1 per cent of 159 copepods (including both adults and copepodid stage V) with an average number of procercoids per copepod of 1.9 (1

to 6). The infection data broken down for the two sexes and for the adult and copepodid stage V are shown in Table 1.

The average numbers of procercoids in the copepodids are higher than in the adult copepods, possibly suggesting a greater susceptibility to infection in the copepodid stage V.

Procercoids developed to the cercomer stage with calcareous bodies apparently equally well and at a similar rate in both the copepodids and the adults, reaching the fully formed condition in 11–12 days. As in *D. piscinae* the development followed the sequence of events outlined by Janicki and Rosen (1917) and by Vogel (1930). The oldest procercoids were observed 16 days after infection.

Diaptomus mississippiensis Marsh.

Specimens of this species were provided by Dr. M. S. Ferguson, who collected them near Atlanta, Georgia. Procercoids developed in 91.0 per cent of 89 copepods infected in Georgia, with an average number of procercoids per copecod of 3.2 (1 to 10). The breakdown of infection rates by sexes is shown in Table 1. Procercoids also developed in a second group of 29 *D. mississippiensis* infected in Boston. Of these only 27.6 per cent contained procercoids. The lighter infection may have been caused by the small numbers of coracidia to which these copepods could be exposed at the time.

Diaptomus albuquerquensis Herrick.

Dr. M. S. Ferguson has kindly given me permission to report that he has infected this species with coracidia of *D. latus*. In many individuals collected at Atlanta, Georgia, he observed development of the procercoids over the first 3 days. This species seemed to be as susceptible to infection as *D. mississippiensis*. The ease with which this species became infected would suggest that normal development of the procercoids would take place.

Eurytemora affinis Poppe.

This species was collected in limited numbers from the Charles River at Boston, Massachusetts. Procercoids developed in 83.3 per cent of 24 individuals, with an average number of procercoids per copepod of 5.8 (1 to 30). The breakdown of infection data by sexes is indicated in Table 1.

Because of the great difficulty experienced in maintaining *E. affinis* in the laboratory, data on the late development of the procercoids were difficult to obtain. However, in one copepod 17 days after infection 3 fully formed procercoids with cercomers and calcareous bodies were seen. None of the remaining copepods survived beyond 10 days after infection.

Other species of copepods.

Small numbers of several other species of copepods from various localities in the vicinity of Boston were subjected to similar exposure to coracidia. None became infected, however, although control *Diaptomus* developed procercoids. These species were *Cyclops ater* Herrick (19 females), *C. fuscus* (Jurine) (100 females), *C. bicuspidatus thomasi* Forbes (34 females and 7 males), and *Canthocamptus staphylinoides* Pearse (20 females). In addition, Dr. M. S. Ferguson has reported in cor-

respondence that he was unable to infect 2 to 3 dozen *Osphranticum labronectum* Forbes or 3 to 4 dozen *Diaptomus birgei* Marsh from Athens, Georgia.

DISCUSSION

It is difficult to categorize all the copepods used in experimental *D. latus* infections according to the system of Michajlow (1938). This is because (1) earlier investigators gave insufficient data on the degree of infection or the rate of development in the copepods and (2) usually only small numbers of copepods were used in the experiments. However, certain species may be indicated as falling in group 5: *Diaptomus gracilis, D. graciloides, D. oregonensis, D. vulgaris, D. sicilis, D. siciloides, D. piscinae, D. sanguineus, D. mississippiniensis, D. albuquerquensis,* and *Eurytemora affinis.*

In respect to Michajlow's categories the position of *Cyclops strenuus* is not clear. The small number of procercoids in the experimental infections by Janicki and Rosen (1917) and the apparent differences in susceptibility of this species found by Vogel (1930) would seem to indicate that the susceptibility of *C. strenuus* to *D. latus* infections is of a low grade. Vergeer (1936) and Michajlow and Wierzbicka (1935) have considered *C. strenuus* as a secondary rather than as a principal host. It is not known whether or not procercoids of North American *D. latus* (possibly, according to Wardle (1935), a race physiologically different from European worms) will develop in *C. strenuus.*

Table 2 summarizes the known copepod hosts of *Dibothriocephalus latus.*

The ease with which most of the species of *Diaptomus* thus far investigated become infected and the general resistance of *Cyclops* to infection seem to indicate a generic specificity.

No significant sexual differences in copepods in respect to infection or rate of development of the procercoids, such as those mentioned by Michajlow (1938) in *Triaenophorus* and Gailliard and Ngu (1946) in *D. mansoni*, have been observed in *Dibothriocephalus latus.*

Copepodid stage V of *Diaptomus sanguineus* apparently can be infected somewhat more readily than adults and serves as readily as adults for the development of procercoids. Vogel (1930) noted that younger, sexually immature *Cyclops strenuus* could be more easily infected with *D. latus* than mature individuals.

The susceptibility of the euryhaline copepod *Eurytemora affinis* to infection with *D. latus* suggests the possibility that marine fish might have an opportunity to become infected with the plerocercoids of this tapeworm.

CONCLUSIONS

1. *Diaptomus piscinae* Forbes, *D. sanguineus* Forbes, *D. mississippiensis* Marsh, and *D. albuquerquensis* Herrick have been experimentally infected with coracidia of *Dibothriocephalus latus.* Procercoids developed to the fully formed condition in the first three species, and early development occurred readily in the fourth. This brings to 10 the number of species of *Diaptomus* known to serve as experimental first intermediate hosts of *D. latus.*

2. Procercoids developed readily also in the euryhaline copepod *Eurytemora affinis* after experimental infection.

3. *Cyclops ater* Herrick, *C. fuscus* (Jurine), *C. bicuspidatus thomasi* Forbes,

Table 2.—*Known copepod hosts of* Dibothriocephalus latus *(L.)*

Readily infected experimentally and presumably potential natural hosts		Low % of infection experimentally or arrested development of procercoids		No infection or if coracidia ingested no procercoids develop	
Species	Investigator	Species	Investigator	Species	Investigator
Diaptomus gracilis Sars	Janicki & Rosen (1917), Vogel (1930), Michajlow & Wierzbicka (1935)	*Cyclops strenuus* Fischer	Janicki & Rosen (1917), Redlich (1925), Vogel (1930), Michajlow (1932), Michajlow & Wierzbicka (1935), ?Skvortzov & Talysin (1940)	*Cyclops viridis* (Jurine)	Janicki & Rosen (1917), Redlich (1925), Vogel (1930)
Diaptomus graciloides Lilljeborg	Redlich (1925)	*Cyclops vicinus* Uljanin	Michajlow & Wierzbicka (1935)	*Cyclops oithonoides* Sars	Janicki & Rosen (1917)
Diaptomus oregonensis Lilljeborg	Essex (1927)	*Cyclops vicinus kikuchii* Smirnov	Michajlow & Wierzbicka (1935)	*Cyclops macrurus* Sars	Janicki & Rosen (1917)
Diaptomus vulgaris Schmeil	Vogel (1930)	*Cyclops serrulatus* Fischer	Michajlow & Wierzbicka (1935)	*Cyclops vernalis* Fischer	Janicki & Rosen (1917)
Diaptomus sicilis Forbes	Magath & Essex (1931)	*Cyclops brevispinosus* Herrick	Essex (1927)	*Cyclops leuckarti* Claus	Janicki & Rosen (1917), Essex (1927), Vergeer (1936)
Diaptomus siciloides Lilljeborg	Magath & Essex (1931)	*Cyclops prasinus* Fischer	Essex (1927)	*Cyclops albidus* (Jurine)	Redlich (1925), Essex (1927), Vogel (1930), Vergeer (1936)
Diaptomus piscinae Forbes	present paper	*Cyclops robustus* Sars	Hall (1929)	*Cyclops serrulatus* Fischer	Redlich (1925), Essex (1927), Vogel (1930), Vergeer (1936)
Diaptomus sanguineus Forbes	present paper	*Diaptomus minutus* Lilljeborg	Vergeer (1936)	*Cyclops bicuspidatus* Claus	Essex (1927), Vogel (1930), Michajlow & Wierzbicka (1935), Vergeer (1936)
Diaptomus mississippiensis Marsh	present paper			*Cyclops ater* Herrick	Essex (1927), Vergeer (1936), present paper
Diaptomus albuquerquensis Herrick	present paper			*Cyclops fimbriatus* Fischer	Essex (1927), Vergeer (1936)
Eurytemora affinis Poppe	present paper			*Cyclops americanus* Marsh	Vergeer (1936)
				Cyclops brevispinosus Herrick	Vergeer (1936)
				Cyclops prasinus Fischer	Vergeer (1936)
				Cyclops phaleratus Koch	Vergeer (1936)
				Cyclops fuscus (Jurine)	present paper
				Cyclops bicuspidatus thomasi Forbes	present paper
				Canthocamptus staphylinoides Pearse	present paper
				Osphranticum labronectum Forbes	present paper
				Diaptomus birgei Marsh	present paper

Canthocamptus staphylinoides Pearse, *Osphranticum labronectum* Forbes, and *Diaptomus birgei* Marsh failed to develop procercoids after exposure to coracidia.

4. A somewhat heavier infection occurred in copepodid stage V of *Diaptomus sanguineus* Forbes than in the adults of this species.

5. No significant difference in number infected or degree of infection was observed in the two sexes of infected *Diaptomus*.

6. No apparent differences in the rate of development of the procercoids were observed in the two sexes or in the mature or copepodid stage V instars.

7. Development of the procercoids occurs readily in most of the species of the genus *Diaptomus* thus far investigated. Procercoids develop to a lesser degree or not at all in species of the genus *Cyclops*. Presumably *Diaptomus* is a potential natural host of primary importance, while *Cyclops* is a potential natural host of secondary importance.

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THE GERMINAL DEVELOPMENT IN THE DAUGHTER REDIAE OF AN OPHTHALMO–XIPHIDIOCERCARIA FROM *POMATIOPSIS LAPIDARIA**

D. J. Ameel, W. W. Cort, and Anne Van der Woude

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INTRODUCTION

Ameel (1939) redescribed *Histrionella pomatiopsidis* Stimpson, 1865, an ophthalmo-xiphidiocercaria from *Pomatiopsis lapidaria,* as *Cercaria pomatiopsidis.* He called attention to the similarity of this species to cercariae belonging to the family Allocreadiidae. In fact, recent work indicates that this family should include only species with ophthalmo-xiphidiocercariae (Hopkins, 1934; Cable and Hunninen, 1942). The structure of cercariae of this type, especially the presence of a stylet and stylet glands, suggests that this family is related to the Plagiorchioidea. We might go even further and suggest the hypothesis that this group on account of the presence of eye-spots in the cercariae and their development in rediae is more primitive than the Plagiorchioidea, and may be close to the ancestral type from which the true plagiorchiids developed. This possibility made it of special interest to determine whether the germinal development in the daughter rediae of *Cercaria pomatiopsidis* showed any resemblance to that in the daughter sporocysts of the plagiorchiids.

During the summer of 1948 we had the opportunity of examining 1282 specimens of *Pomatiopsis lapidaria* collected near Ann Arbor, Michigan, of which 37 or about 3.0 per cent were infected with *C. pomatiopsidis.* Most of the infections were mature or old but a few immature daughter rediae were available for study.

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Description of Figures

Germinal material in daughter rediae of *Cercaria pomatiopsidis.*

Fig. 1. Immature daughter redia, 0.30 × 0.09 mm, showing small germinal mass with unicellular and multicellular components.

Fig. 2. Posterior half of immature daughter redia, 0.36 × 0.075 mm, showing germinal mass with unicellular and multicellular components pushed to one side by large embryos.

Fig. 3. Posterior end of immature daughter redia, 0.47 × 0.09 mm.

Fig. 4. Posterior end of immature redia showing germinal mass with large multicellular components.

Fig. 5. Posterior end of almost mature daughter redia 0.24 × 0.10 mm; largest cercaria has eye-spots.

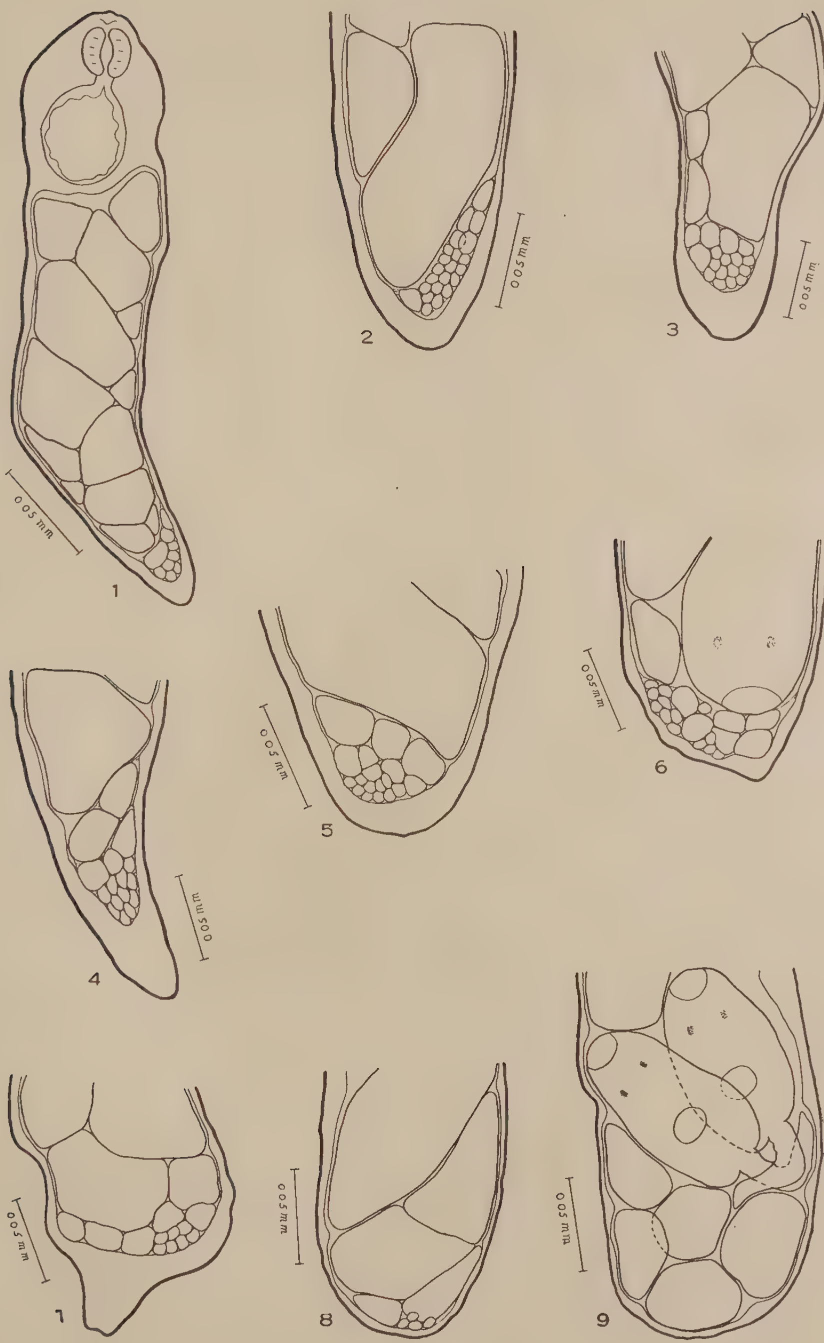
Fig. 6. Posterior end of mature redia, 0.63 × 0.105 mm, with unusually large germinal mass.

Fig. 7. Posterior end of mature redia, 0.60 × 0.10 mm; number of germinal cells reduced.

Fig. 8. Posterior end of mature redia 0.83 × 0.15 mm; showing great reduction in number of germinal cells.

Fig. 9. Posterior end of mature redia 0.78 × 0.12 mm, showing no trace of germinal cells of germinal mass; there were 16 cercariae with eye-spots in this redia.

PLATE I



GERMINAL DEVELOPMENT

In the smallest daughter redia studied, attached at the posterior tip of the body cavity, there was a small germinal mass which had small numbers of both unicellular and multicellular components (Fig. 1). A rather small number of cercarial embryos filled the body cavity of this redia, none of which were mature enough to show eyespots. However, even at this early stage the embryos were not arranged in order of size with the largest anteriad as in the amphistomes and notocotylids (Cort, Ameel, and Van der Woude, 1948). In older rediae, embryos of all sizes were mixed together in the body cavity, and mature freely moving cercariae were frequently in contact with the germinal mass (Fig. 6). Figures 2, 3, and 4 show the germinal masses in larger immature daughter rediae. They are composed of groups of germinal cells with small embryos (multicellular components) attached either in front or to the sides. In figure 6 the germinal mass appears to be slightly larger and more complex. Figures 7 and 8 show a great reduction of the germinal cells with the disappearance of distinct germinal masses in mature daughter rediae, and in the mature redia shown in figure 9 all the germinal cells have long since developed into embryos.

DISCUSSION

Although the daughter rediae of *C. pomatiopsidis* have germinal masses with both unicellular and multicellular components they are small, have few components, and are completely used up by the production of embryos about the time the rediae reach maturity. Thus the period of multiplication of germinal cells is restricted, and therefore rather small numbers of individuals are produced. In counts of daughter rediae in 20 mature and old infections the numbers ranged from 24 to 128 with an average of 74. While counts of cercariae escaping from individual infections were not made, the number appeared to be small, and only a comparatively few cercarial embryos were present in the daughter rediae. This contrasts with the very large numbers of cercariae characteristically produced in plagiorchiid infections. In the species of that group in which the germinal development has been studied (Cort and Olivier, 1943; Cort and Ameel, 1944) the great production of individuals is brought about both by the development of large numbers of daughter sporocysts in the highly modified mother sporocyst and by the long-continued division of the germinal cells in the large germinal mass that is present in each daughter sporocyst. Therefore, there is little similarity between the mechanism of germinal development in *C. pomatiopsidis* and the plagiorchiids. This raises the question of whether the condition in *C. pomatiopsidis* is really characteristic of the family Allocreadiidae. While this cannot be answered until further studies are made of other representatives of this group, the possibility must be considered that the limited reproductive capacity in this species is a secondary modification related to the small size of the snail intermediate host.

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PHOCITREMOIDES OVALE N. GEN., N. SP. (TREMATODA: OPISTHORCHIIDAE), WITH OBSERVATIONS ON ITS LIFE CYCLE

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INTRODUCTION

Maxon and Pequegnat (1949) described two, eyespotted and pleurolophocercous, cercariae which they designated Pleurolophocercous I and II from the marine snail, *Cerithidea californica* Haldeman, collected in upper Newport Bay, California. Their Pleurolophocercous I probably is the cercaria of *Parastictodora hancocki* whose characters and life cycle were described by Martin (1950b). Their Pleurolophocercous II is here reported as a new genus and species of OPISTHORCHIIDAE Braun, 1901.

MATERIALS AND METHODS

Living and fixed entire specimens and serial sections were used. Whole mounts were stained with paracarmine and mounted in Permount. The cercariae were killed by forcibly ejecting them from a pipette into cold Bouin's solution. Living material was used to determine the excretory patterns.

OBSERVATIONS

Phocitremoides n. g.

Generic diagnosis: Small, pyriform to oval trematodes. Cuticula scaled over most of body. Suckers nearly equal in size, well-developed. Prepharynx short, pharynx well-developed, esophagus short, ceca slender and extending to posterior margin of testis. Testis single, large, in posterior one-third of body. Seminal vesicle large, bipartite, and C-shaped. Ovary oval, immediately anterior to testis. Laurer's canal present. Vitellaria between acetabular and post-testicular levels. Seminal receptacle well-developed, close to ovary. Uterus mainly intercecal, filling posterior half of body and extending slightly anterior to acetabulum. Eggs relatively large, yellow, operculate, without polar filaments. Genital sac spacious, thick-walled, immediately anterior to acetabulum. Excretory vesicle U-shaped, excretory pattern $2[(2+2+2)+(2+2+2)] = 24$. Remnants of eyespots may be present.

Phocitremoides ovale n. sp.

(Figure 1)

Specific diagnosis: With characters of genus. Body oval to pyriform, measuring 0.223–0.266 (mean 0.231 mm) in length and 0.142–0.184 (mean 0.160 mm) in maximum width. These measurements are based upon twenty-five stained and mounted specimens. Cuticula armed with relatively large scale-like spines (Fig. 2) over anterior half of body, except for a ventral area between the suckers, but decreasing in size and finally disappearing toward the posterior end.

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PLATE I

All drawings made with the aid of a camera lucida unless otherwise stated.

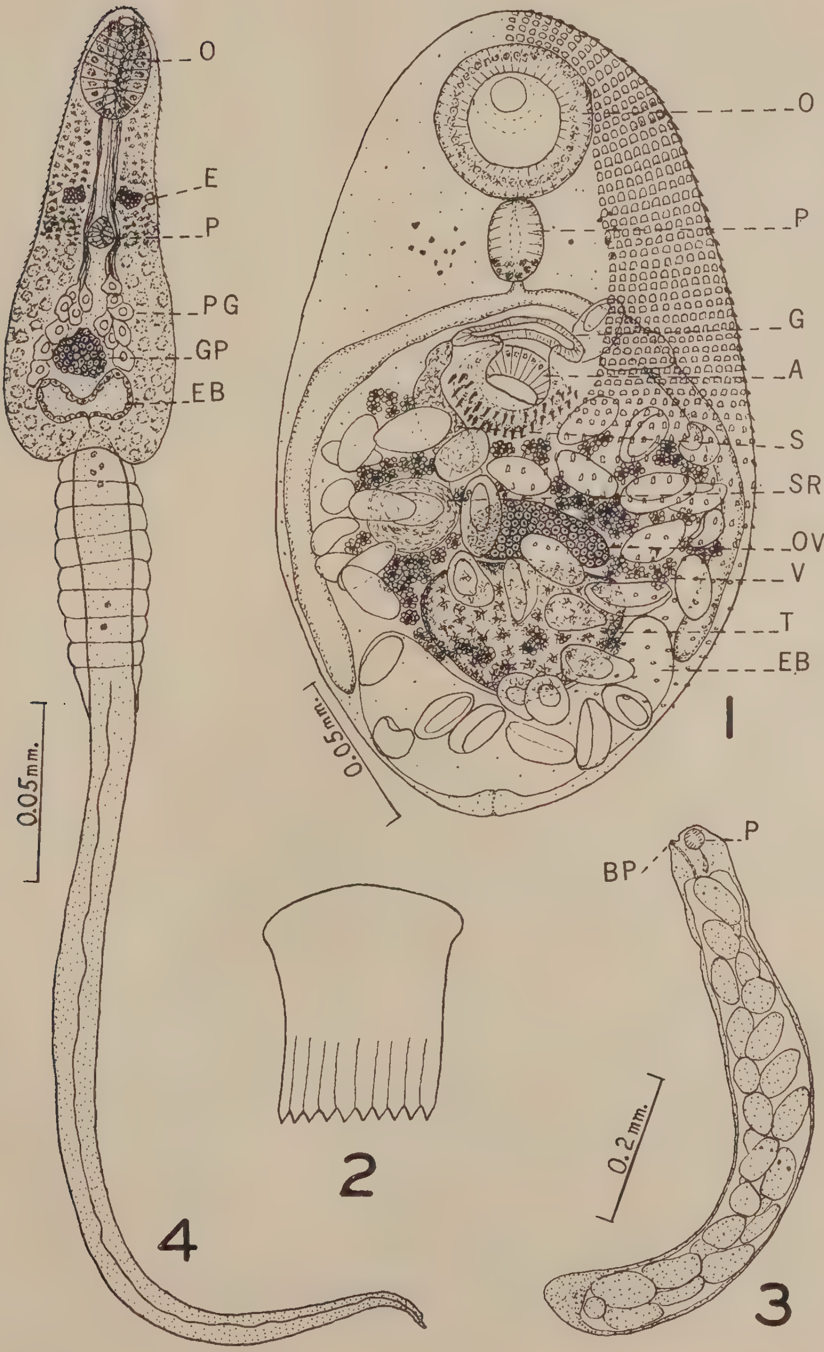
Fig. 1. Adult *Phocitremoides ovale*.

Fig. 2. Diagram of serrate scale of adult.

Fig. 3. Redia.

Fig. 4. Cercaria.

PLATE I



Two rows of narrow pointed spines occur along the margin of the posterior half of the ventral sucker. Oral sucker circular to oval, subterminal, averaging 0.047 mm by 0.054 mm. Prepharynx short, followed by an oval, muscular pharynx averaging 0.019 mm by 0.024 mm. Esophagus usually shorter than pharynx. Ceca narrow, and extending laterally and posteriorly to terminate at the level of posterior margin of testis. Acetabulum well-developed, frequently retracted into body, averaging 0.039 mm by 0.046 mm. Genital atrium thick-walled, transversely elongate, immediately anterior to the acetabulum. Seminal vesicle weakly divided into two parts, frequently bent upon itself, located slightly to right of acetabulum. The vas deferens leads from the large, single testis which is located in the posterior one-third of the body to the seminal vesicle. Testis oval, averaging 0.043 mm by 0.056 mm. Ovary oval, immediately anterior to testis, averaging 0.023 mm by 0.033 mm. Laurer's canal present; seminal receptacle oval to spherical, to right of ovary, sometimes dorsally overlapping testis; convolutions of uterus fill posterior half of body and extend slightly anterior to acetabulum. Vitelline follicles dorsal and lateral, between the acetabular and post-testicular levels. Eggs yellow, operculate, without polar filaments, averaging 0.016 mm by 0.026 mm. The older eggs contain miracidia without eyespots, whose cilia move slowly and intermittently before hatching. Excretory bladder weakly U-shaped with horns extending anteriorly to the mid-testicular level. Main collecting ducts arise from anterior margin of vesicular horns, extend anteriorly to acetabular level where they divide into anterior and posterior branches, each of which drains three sets of paired flame cells. An anastomosing duct connects right and left excretory branches immediately posterior to the pharynx.

Develops experimentally in the small intestines of chicks and cats. Metacercariae encyst on under sides of scales of the marine fishes, *Atherinopsis californica* Girard and *Fundulus parvipinnis parvipinnis* (Girard). Cercariae and rediae develop in the marine snail, *Cerithidea californica* Haldeman.

Type locality: Coast of Southern California.

Type specimen: *Phocitremoides ovale,* deposited as number 502, Hancock Parasitology collection.

Redia

(Fig. 3)

The rediae are tubular structures which develop in the digestive gland of *Cerithidea californica.* Sixteen rediae varied in length from 0.671 mm to 0.956 mm (mean 0.797 mm) and 0.089 mm to 0.134 mm (mean 0.104 mm) in maximum width. Near the anterior end is a small, almost spherical pharynx which varied from 0.021 mm to 0.026 mm (mean 0.024 mm) in length and 0.020 mm to 0.025 mm (mean 0.022 mm) in width. The pharynx opens into a small saccular gut. Each redia is thin-walled except at the posterior end where there is a mass of cells which gives rise to germ balls. All sixteen rediae contained germ balls and well-developed cercariae. Near the anterior end of each redia is a birth pore.

Cercaria

(Figs. 4, 5, 6)

The cercaria is biocellate, monostomous, and pleurolophocercous. The body varies from 0.083 mm to 0.182 mm (mean 0.111 mm) in length and 0.026 mm to 0.051 mm (mean 0.040 mm) in maximum width. These measurements are based upon twenty-four stained and mounted specimens. The anterior half of the body cuticle is spined. Anterior to the mouth is a series of four rows of spines, the proximal being the largest and having the points of the spines directed toward the

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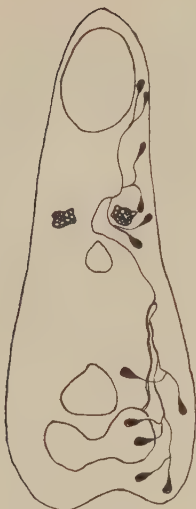
Plate II

Fig. 5. Outline of cercaria showing flame cell pattern.
Fig. 6. Diagram of cercarial mouth and associated spines.
Fig. 7. Twenty-four-hour-old metacercariae on scale of *Atherinopsis californica.*
Fig. 8. Fully-developed metacercaria.



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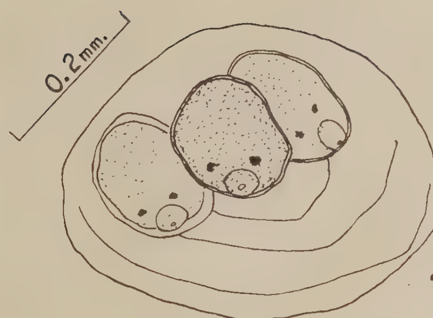

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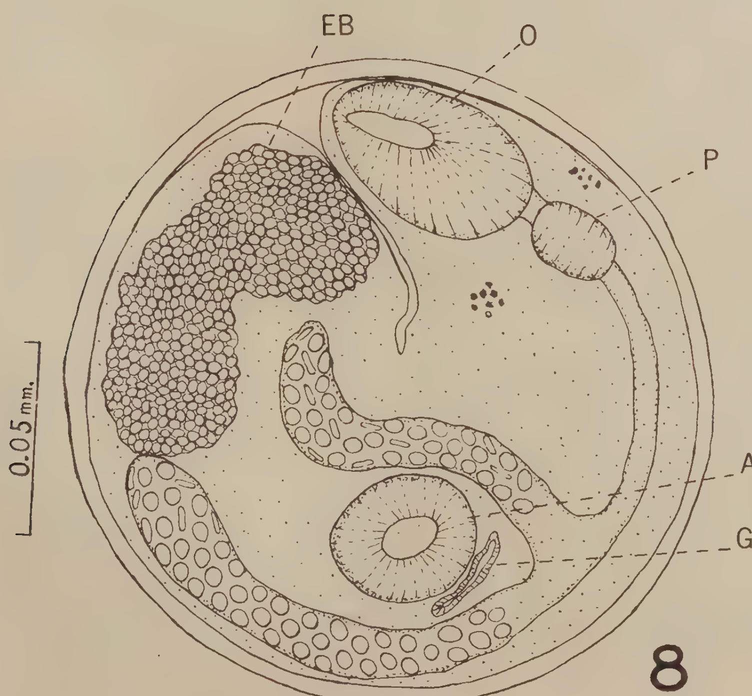

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mouth opening. This row consists of four spines, the others of four, five, and six, respectively (Fig. 6). These spines are located on the ventral side of a protrusible cone which also bears the openings of the penetration gland ducts, the latter divided near their exits into four bundles arranged in a 3-4-4-3 pattern resembling the arrangements of these ducts in the cercariae of *Euhaplorchis californiensis* Martin, 1950 and *Parastictodora hancocki* Martin, 1950. The cell bodies of the fourteen penetration glands are located in the posterior half of the body partially surrounding the genital primordium. A distinct incomplete ring partially surrounding the mouth as in the cercariae of *Euhaplorchis californiensis* and *Parastictodora hancocki,* is lacking. The oral sucker can be retracted into the body; it measures 0.017–0.020 mm (mean 0.019 mm) by 0.018–0.025 mm (mean 0.022 mm). The prepharynx extends from the oral sucker to the pharynx which is located a short distance posterior to the eyespots. The pharynx varies from 0.005–0.007 mm in diameter. The rest of the digestive tract is undeveloped. The posterior three-fourths of the body is glandular and, in living specimens, a light brown. The excretory bladder is weakly U-shaped. From its arms the main collecting ducts extend anteriorly to the mid-body region where they divide into anterior and posterior branches, each of which drains three pairs of flame cells. The excretory pattern is the same as that of the metacercaria and adult (except for the anastomosis) and is represented by the formula $2[(2+2+2)+(2+2+2)]=24$.

The tail is long and slender, measuring 0.350–0.425 mm (mean 0.380 mm) in length and 0.028–0.042 mm (mean 0.035 mm) in maximum width. The proximal portion of the tail has a cuticular sheath, approximately 0.082 mm long on the average, which may be annulated and sometimes appears to be only on one side. Near the posterior end of the cuticular sheath, a dorsal fin begins, extends posteriorly around the tip of the tail, and, as the ventral fin, continues anteriorly to near the level of its dorsal origin.

Metacercaria
(Figs. 7 and 8)

The cercariae shed their tails and encyst as metacercariae on the under sides of the scales of the jack smelt, *Atherinopsis californica* Girard, and the Southern California killifish, *Fundulus parvipinnis parvipinnis* (Girard). Figure 7 shows a scale of *Atherinopsis californica* after a twenty-four-hour exposure to cercariae. In such experimental infections, as many as seven metacercariae have been found on one scale. In older metacercariae, such adult structures as the acetabulum, genital atrium, and complete digestive tract, are found. The metacercariae have proportionately longer prepharynges and esophagi than do the adults. The intestinal ceca and the excretory bladder contain circular concretions.

When experimentally infected fishes were fed to newly-hatched chicks and to cats, egg-producing adults were recovered from the small intestine after a lapse of four days. The worms were located in the anterior portion of the small intestine of the cat and from one-half inch anterior to the attachment of the yolk sac to the beginning of the large intestine in the chick. A total of four cats and twelve chicks were fed infected fishes and all developed infections. Two cats and six chicks used as controls were negative.

DISCUSSION

The genus *Phocitremoides* resembles the genus, *Phocitrema* Goto and Ozaki, 1930, in the general arrangement of major organs but differs from it in having one testis, vitellaria not extending posterior to the testis, and a spacious genital atrium, whereas *Phocitrema* has two testes, vitellaria extending posterior to the testes, and a small genital atrium. Unfortunately, the excretory bladder of *Phocitrema* was not described. *Phocitrema fusiforme* Goto and Ozaki, 1930 was obtained from the intestine of a seal, *Phoca hispida* Schreber, which died in the Hanayashi Zoological Garden, Tokyo, Japan. Probably *Phocitremoides ovale* can develop in seals as well as in piscivorous birds.

Although a gonotyl is lacking in the genus *Phocitrema,* it otherwise bears considerable resemblance to members of the family Heterophyidae and was included in this family by Price (1932). However, Price (1940) placed this genus in the subfamily Phocitrematinae Yamaguti, 1933 of the family Opisthorchiidae Braun, 1901, but again stated that it differed from the Heterophyidae only in the absence of a gonotyl. Price (1940) included the genus *Witenbergia* Vaz, 1932 in the Phocitrematinae but stated that it too bore great resemblance to the Heterophyidae. *Witenbergia witenbergi* Vaz, 1932 was recovered from the small intestine of a fresh-water fish, *Pseudoplatistoma tigrinum,* collected in the Piracicaba River, São Paulo, Brazil. The genus *Witenbergia* differs from *Phocitrema* mainly in the more elongate prepharynx and esophagus and in the more posterior extent of the uterus in the former.

The genus *Phocitremoides* is placed provisionally in the subfamily Phocitrematinae, for although the vitellaria do not extend posterior to the testis, they do overlap it. The cercaria of *Phocitremoides ovale* is similar to that of *Opisthorchis felineus* as described by Vogel (1934) and lends further support to the placement of the Phocitrematinae in the Opisthorchiidae.

SUMMARY

A new genus and species of trematode, *Phocitremoides ovale,* belonging to the family Opisthorchiidae, is described.

The pre-metacercarial stages develop in the digestive gland of the marine snail, *Cerithidea californica* Haldeman. The rediae are tubular and have a birth pore near the anterior end. The cercariae are biocellate, monostomous, and pleurolophocercous. They have a flame cell pattern represented by the formula $2[(2+2+2)+(2+2+2)]=24$ which is retained through the adult stage.

The metacercariae encyst on the under sides of the scales of the jack smelt, *Atherinopsis californica* Girard and the Southern California killifish, *Fundulus parvipinnis parvipinnis* (Girard).

Egg-producing adults were obtained after a lapse of four days following the feeding of infected fishes to cats and newly-hatched chicks. They were found in the anterior small intestine of the cat and from a short distance anterior to the yolk sac attachment to the beginning of the large intestine in the chick.

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Explanation of Plates

Abbreviations

A	acetabulum	GP	genital primordium	S	seminal vesicle
BP	birth pore	O	oral sucker	SR	seminal receptacle
E	eyespot	P	pharynx	T	testis
EB	excretory bladder	PG	penetration gland	V	vitellaria
G	genital atrium				

STUDIES ON THE LIFE HISTORY OF *EURYTREMA PANCREATICUM* JANSON, 1889*

C. C. Tang

INTRODUCTION

The elucidation of life cycles of the trematodes belonging to the subfamily Dicrocoeliinae Looss, 1899, is a comparatively recent event, although since the time of Leuckart (1888) and Piana (1882) experimenters have tried to infect snails with eggs of *Dicrocoelium dendriticum*. The work of Vogel (1929) and Cameron (1931) identified *Cercaria vitrina* v. Linstow, 1887 as the larval stage of *D. dendriticum*, and involved land snails as the intermediate hosts. Mattes (1933 and 1936) showed the complete development of this trematode in its molluscan intermediary, and Neuhaus (1936, 1938) discovered the composite cysts of the cercariae and the method of infection of the definitive host. Thus the life cycle of *D. dendriticum* was made known. Influenced by these investigations, studies on the life cycles of other dicrocoeliid species soon followed. The investigations of Denton (1944 and 1945) on the life cycle of *Eurytrema procyonis* [= *Concinnum procyonis* (Denton, 1942) Travassos, 1944] and *Brachylecithum americanum*, and of Maldonado (1945) on that of *Platynosomum fastosum* brought important results revealing considerable differences in the habits of different species in this group. The involvement of an insect host for *B. americanum* and a lizard for *P. fastosum*, in contrast to *D. dendriticum* which requires no second intermediate host, indicated the varied adaptation of this most interesting group of trematodes and encouraged further researches on the life cycles of additional species. The present investigation on the biology of *Eurytrema pancreaticum* was started in Shao-wu, northwestern Fukien in 1944, and was later continued in Foochow, where the parasite is very common. In 1948, the writer was able to obtain both experimental and natural infections of snail hosts, enabling him to study the intra-molluscan phase of the development of this trematode. He was, however, unable to produce an experimental infection in goats through feeding them with mature second generation sporocysts secured from infected snails.

Eurytrema pancreaticum has been recorded from the pancreatic and biliary passages of various animals such as the ox, water buffalo, sheep, and goat in the Orient. It is also common in the pancreatic duct of hogs in Hongkong and occurs less commonly in camels in North China and in the monkey, *Macaca syrichta fascicularis*. This common trematode was recorded once from man in South China by Castellani and Chalmers (Faust, 1949, p. 206). Its distribution is extensive, being reported in China, Japan, Indo-China, India, Malay Archipelago, and the Philippines.

EXPERIMENTAL AND NATURAL INFECTIONS

Adult worms from both goats and cattle were secured from slaughter houses. Eggs were dissected from the uteri of mature worms. Those eggs, dark-brown in

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* Contribution from the Institute of Zoology and Botany, Fukien Academy, Foochow, China, and the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University. The writer wishes to express his grateful indebtedness to Dr. W. W. Cort for his advice and help in the preparation of the manuscript. He also wishes to express his sincere thanks to Mr. Tucker Abbott for the identification of the molluscan hosts.

color and containing mature miracidia, were used in the feeding experiment. Six species of snails, *Lymnea (Radix) plicatula* Benson, *Hippeutes cantori* Benson, *Segmentina hemisphaerula* Benson, *Succinea chinensis* Pfeiffer, *Bradybaena similaris* Férussac and *Cathaica ravida sieboldtiana* Pfeiffer were used for experimental laboratory hosts. The eggs of *E. pancreaticum* hatched in the gut of all six species of snails, but development proceeded only in the last two species, *B. similaris* and *C. ravida sieboldtiana.* Only laboratory-bred snails of these two species were used for experimental infections. They were cultured in large earthen jars with layers of gravel, loose earth, dry leaves and twigs following the method described by Krull (1937). The moisture of the culture-jar was adjusted by adding to or reducing the amount of water at the bottom. Snails were fed once every three or four days.

Eggs were smeared on the surface of lettuce leaves and fed to groups of 25 to 50 snails in each experiment. Droppings of snails were then examined for the sign of hatching as shown by the opening of the operculum. Feeding experiments with *B. similaris* and *C. ravida sieboldtiana* performed in 1944 were considered negative because the writer failed to recognize the early developmental stages of the mother sporocysts embedded in the tissue of the host. In 1948, when laboratory-bred snails were used and the method of culturing land snails adopted, the prolonged incubation of the parasite made it possible to find the daughter sporocysts. These findings suggested the necessity for further study. A few positive experiments are recorded as follows:

A batch of 53 laboratory-bred snails were fed with eggs of *E. pancreaticum* on February 19, 1948. The snails used consisted of both *B. similaris* and *C. ravida sieboldtiana* of sizes varying from 3 to 8 mm in diameter. About 30 snails died during the first few days after the start of the experiment and some of them continued to die off each week. Of 13 snails left, 12 ultimately were examined with positive results. Certain of these were dissected on May 28 and others on June 18; mature mother sporocysts were found in the first few snails and daughter sporocysts in the second series. Some snails lived till the beginning of August when they began to expel mature daughter sporocysts. Those dissected on August 3, 164 days after infection, were found to contain numerous fully mature daughter sporocysts packed in the respiratory chamber.

Two more small batches of young snails, about 5 to 8 mm in diameter, were infected experimentally on May 31 and July 2. They were dissected on June 26 and August 5, respectively, and different stages of mother sporocysts were found. In our experiments, all control snails reared under the same laboratory conditions and fed with lettuce were free from infection.

The writer finally succeeded in finding natural infections in *B. similaris* (Fig. 10) and *C. ravida sieboldtiana,* which actually serve as the intermediate hosts of *E. pancreaticum* in cattle and goats in the Fukien region, and probably in other parts of China. On Nantai Island, situated at the southern part of Foochow, positive snails were found near a dairy house. Fecal specimens from individual cows in this dairy showed eggs of *E. pancreaticum.* Of 664 snails collected from a citrus garden near the dairy, 21 (3.1%) were positive. Snails collected from places surrounding a cow-manure pit were also positive, while 816 snails collected from other areas, where they had no chance of contact with cow's manure, were all negative. In another series of examinations, positive snails were found in the door yard of a house,

where the dung of one infected cow had been deposited as she was driven there every day to give milk to the people in the neighborhood. In Foochow the infected cows apparently acquired their infection locally, as they were born and raised in the same dairy house. The epidemiological findings as well as the positive laboratory infection of these two species of land snails show that they are the usual intermediaries of *E. pancreaticum* in this region.

THE SNAIL INTERMEDIATE HOSTS

Both *B. similaris* and *C. ravida sieboldtiana* are widely distributed species. The former has been transported by man to many tropical and subtropical areas, including Southern China, India, Mauritius, East Indies, and the Hawaiian Islands. The genus *Cathaica* is limited to China and Central Asia south to India. While their distribution coincides with that of *E. pancreaticum,* other closely related species of land snails must also be involved in North China and many other regions where the parasite is present. Often the shell of *B. similaris* is marked with a single spiral brown band. Not all specimens, however, have this character. This species and *C. ravida sieboldtiana* belong to the Fruticoicolidae, a family related to the Helicidae, members of which like *Helicella* and *Cochlicella,* and also *Zebrina* and *Chondrina* of the family Vertiginidae, have been implicated as the first intermediate hosts of *D. dendriticum*. Copulation of the snails takes place in the early spring. Eggs are laid during April and May in the loose soil or under a protecting covering in a moist environment. They are spherical in shape, pearly white in color and about 2 mm in diameter. These eggs must be kept under a condition of high humidity. When they are in contact with dry air they explode. Young snails just hatched are pale-colored, and are about 2 mm in length. They can be reared on a diet of lettuce leaves. Snails attaining 5 mm in diameter are suitable for infection experiments; young snails are easily infected but the mortality is very high.

EGG AND MIRACIDIUM

The egg of *E. pancreaticum* is operculated, thick-shelled, and measures 0.041–0.055 mm in length and 0.028–0.038 mm in width (average 0.046 by 0.032 mm). The edges of both the operculum and the opening of the shell have indentations, allowing them to fit together tightly (Fig. 1, A). The immature eggs are lighter in color, while the mature ones become dark-brown (Fig. 1, B). The fully developed miracidium inside the egg shell shows no movement. Its anterior end invariably is directed toward the operculum of the shell. The egg does not hatch unless it is swallowed by a mollusc. Apparently the hatching of the egg is not related to the specific host since experiments show that different species of snails can induce the hatching, but the development can proceed only in the specific host.

Sometimes the miracidium can be forced out of the shell by applying pressure to the cover glass. The miracidum thus hatched shows considerable motility. The cilia, especially those on the anterior part, beat actively. Structures in the recently hatched miracidium can be observed most clearly. The miracidium (Fig. 1, C) measures about 0.032 by 0.028 mm. It is armed on its anterior end with a stylet, which can be pushed forward or withdrawn. The stylet is about one-fourth the length of the whole body and is inserted in front of the central nerve mass. There are two rows of ciliated epidermal plates, but the number in each row was not de-

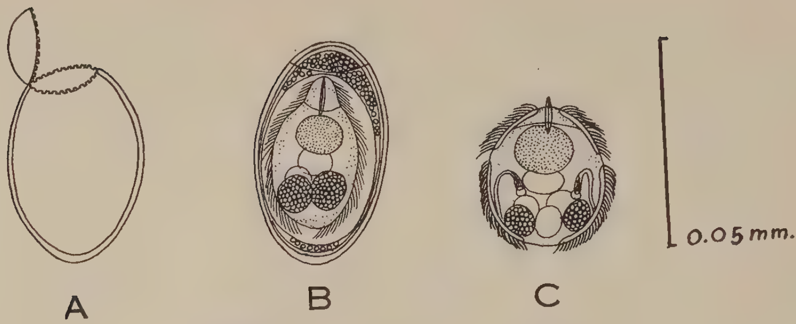

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FIG. 1. (A) Egg-shell, (B) mature egg, and (C) miracidium of *E. pancreaticum.*

FIG. 2. Young mother sporocyst of *E. pancreaticum* 26 days after the penetration of miracidium into the snail host.

FIG. 3. A portion of a mature mother sporocyst dissected from the digestive gland of the snail host.

termined. The anterior and posterior tips of the body, as well as a zone in front of the equatorial region, are non-ciliated. Two flame cells were observed, one on each side of the body. The excretory tubules leading from them bend posteriorward. Two oval vesicles containing numerous refractile granules are oppositely placed in the hind part of the body. These two vesicles or granular masses are present in the miracidia of different species of trematodes belonging to the subfamily DICROCOELIINAE such as *B. americanum, D. dendriticum, Olssoniella mosquensis, E. procyonis,* and *P. fastosum.* They are probably present in the miracidia of all species of trematodes of this subfamily, and have been observed by different authors. Leuckart interpreted them to be "the rudiment of a future brood," by which he probably meant "the germinal primordial cells." However, observations made in the present study indicate that they are not the germinal cells of the miracidium. As they are so close to the excretory tubules leading from the two flame cells, the writer is of the opinion that they are probably excretory vesicles. Careful examination revealed no excretory pores. Four large cells are discernible occupying the central and hinder part of the miracidium. They are probably the germinal cells.

The hatching apparently takes place soon after the egg is eaten by the snail host. Eggs with opercula opened were observed in droppings passed by the snails shortly after the eggs were fed. The behavior of the miracidium inside the body of the snail was not observed.

STAGES IN INTERMEDIATE HOST

The mother sporocyst: The mother sporocyst as observed about four weeks after infection is a round mass measuring about 0.092 mm in diameter (Fig. 2). Snails dissected during the fourth week after infection contained many such round masses embedded in the digestive gland. Judging from the position of the early mother sporocyst, it is assumed that the miracidium must have penetrated the mid-intestinal wall (that portion of the alimentary canal turning forward in an acute curvature in the visceral hump). The miracidum comes to lie within the tissue or in the follicle of the digestive gland where it metamorphoses into the mother sporocyst. The mother sporocyst is closely surrounded by fibrous tissue of the host, which probably develops as a reaction to the presence of the parasite. Numerous germinal cells with large nuclei and centrally placed nucleoli are evenly distributed in the mother sporocyst. Such uniform distribution of germinal cells suggests that there may occur a simultaneous development of different individual daughter sporocysts from these cells. However, as the later growth of the daughter sporocyst is traced, considerable differences in size are found. The early development of the mother sporocyst itself is quite slow, especially in the first three to four weeks. The subsequent growth becomes more rapid. In snails 97 days after infection, the mother sporocyst has grown into a many lobed mass in the digestive gland of the host (Fig. 3). At this stage, and even earlier, numerous daughters are already evident. The growth of the mother sporocyst appears to be limited by the space within the follicles of the gland. Such space may also determine the form of its lobes. The mother sporocyst is so closely surrounded by the host tissue that its limits are difficult to determine. It is therefore very difficult to dissect out a whole mother sporocyst without rupturing it. The number of daughter sporocysts produced by a single mother is also very hard to determine. On one occasion a mature mother sporocyst was found attached to

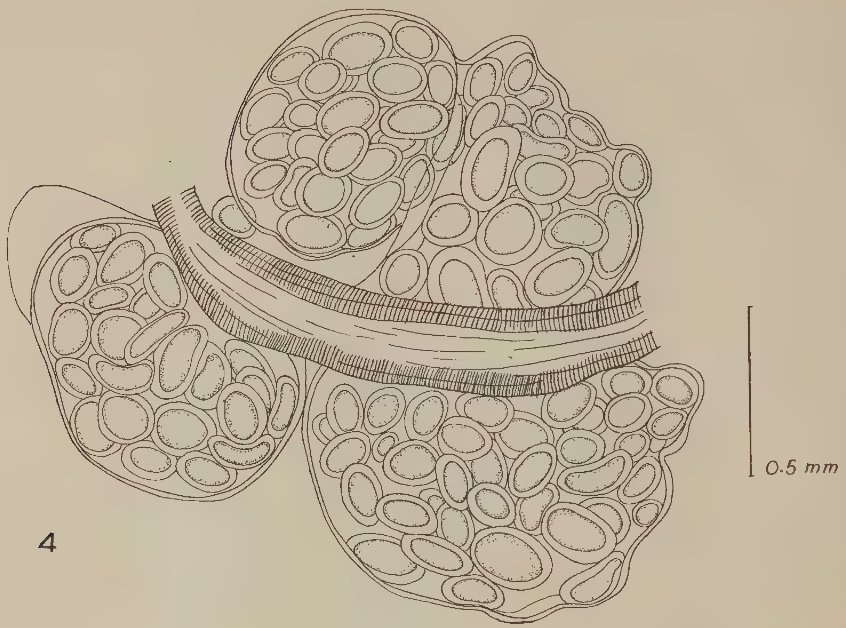

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FIG. 4. A complete mother sporocyst attached to the intestine of the snail host.
FIG. 5. Developing daughter sporocysts showing various stages.

the outer surface of the intestine, where no gland tissue was involved (Fig. 4). This intact mother sporocyst possessed four lobes with thin membranous walls and contained 108 daughter sporocysts. Mature mother sporocysts may attain a diameter of 4 mm. Old mother sporocysts appear gradually to die, leaving the growing daughter sporocysts in their matrix.

The daughter sporocyst: The youngest daughter sporocyst dissected out from the mother was an oval, transparent, sac, with a thick wall and numerous germinal cells inside (Fig. 5). It measured about 0.119 mm by 0.098 mm with a wall from 9 to 23 μ in thickness. On each side of the body an excretory opening was observed which was connected to an excretory tube that divided into three tubules terminating with flame cells. The germinal material consisted of tightly-packed, round, germinal cells filling the whole cavity. Although no inner membrane was observed at this stage, the demarcation between the central mass and the outer somatic wall was very clear. Daughter sporocysts dissected from snails 97 days after infection were still oval in shape. Forty daughters of this stage, measured under pressure of a cover glass, showed an average size of 0.226 by 0.140 mm and a range of 0.119--0.424 by 0.063–0.238 mm. The germinal cells had already changed into germ balls. Cercarial embryos with six to eight cells, enclosed by a thin cellular membrane were observed while the later stages showed larger embryos with more cells. The inner cyst wall (endocyst) is already well developed at this time. Granular substances were found deposited in the space surrounding the central mass and lining the inner surface of the wall. At later stages, long bipolar muscle cells develop in the wall. They are elongate or spindle-shaped with both ends extending into cytoplasmic processes. Their nuclei are large and nucleoli very prominent (Fig. 6). Such cells are able to contract and extend, giving rise to the characteristic movement of the sporocyst. Variation in size of daughter sporocysts is shown in figure 5.

Daughter sporocysts dissected from snails 118 days after infection were more elongate in shape. Many of the smaller ones were, however, still in their former shape and size. At this stage 20 sporocysts were measured: they had an average size of 0.287 by 0.133 mm with a range of 0.114–0.476 by 0.091–0.224 mm. By this time the mother sporocyst has degenerated. The daughter sporocysts lying within the matrix of the mother showed much more activity. Each sporocyst now develops at its anterior end a slender proboscis-like portion, which possibly serves both as an attaching and absorbing apparatus. The space between the two cyst walls increases, and the cercarial embryos become still larger. The movement of the daughters by the contraction of the body wall can be noticed (Fig. 5). During the following stages, variation in size of the daughter sporocysts becomes more and more evident. Those of larger size move more actively. Gradually more mature ones are found in the anterior part of the host. A daughter sporocyst 1–2 mm in length usually contains cercarial embryos already well-formed showing suckers, a stylet, and a tail. The proboscis-like anterior portion of the daughter sporocyst becomes a hollow tubular structure filled with granules. A space surrounds the whole inner cyst wall. It is now filled with more granules, making it appear dark under transmitted light. The cercariae evidently receive their nourishment through the cyst-walls by osmosis. The excretory system of the sporocyst becomes more complicated. The pores on the lateral walls become larger and more prominent. From each excretory pore three or four main collecting tubules arise, running anteriorly, posteriorly and laterally to supply the different parts of the body. Finer branches of tubules could also be made out. Large collecting tubules draining the excretory fluids to the excretory pore are very prominent and are in general very similar to those described by Maldonado (1945) for the daughter sporocyst of *P. fastosum*.

In snails dissected 165 days after infection, many of the daughter sporocysts

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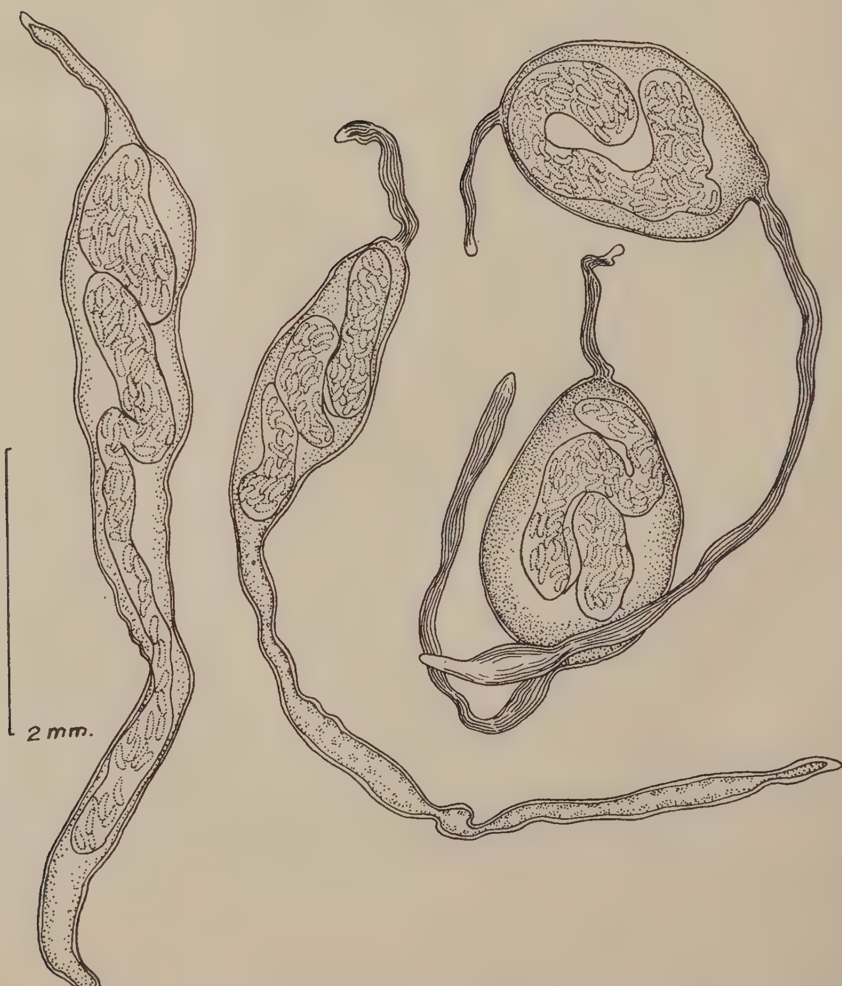

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were fully mature and many had already migrated to the anterior parts of the respiratory chamber, attaching themselves (by means of the anterior slender portions) to the muscles of the head and soft tissues of the mantle near the respiratory aperture. They are white in color, and are often found in clusters. At this stage 30 sporocysts measured from 6.9 mm to 7.9 mm in length and from 0.7 to 1 mm in width. The sporocysts when detached from the host tissues and put in saline solution showed great motility. There is a hollow space in the anterior terminal portion, but no birth pore or birth canal could be made out. By this time all the cercariae were fully developed. The number of cercariae in each daughter sporocyst varied from 144 to 218.

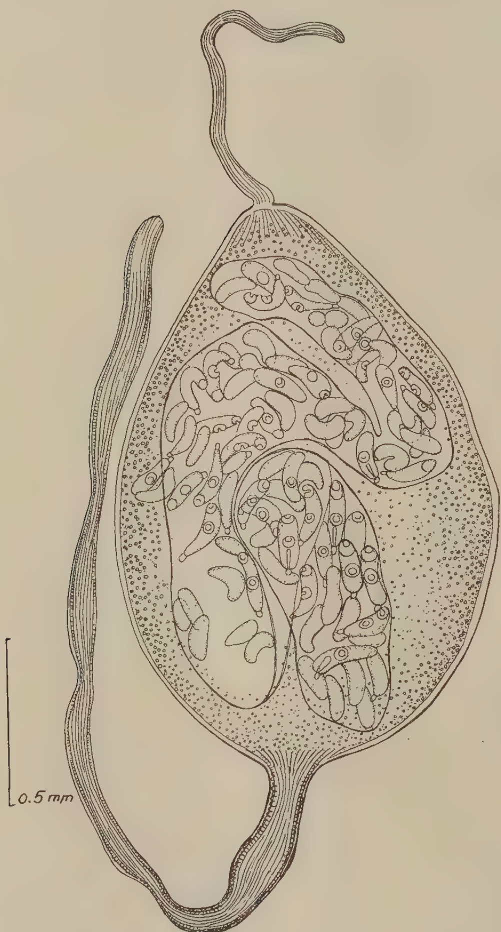
The writer repeatedly observed the expelling of the mature sporocysts from the snails. As the hosts crawl about, the sporocysts are left behind. The expelling takes place both at night and in the day. Such expelled sporocysts always present a peculiar appearance with a portion of the anterior third of the body swollen into a round or oval sac, and with the short anterior terminal portion as well as the rest of the hinder part of the body reduced to thread-like structures. The whole sporocyst appears like a glistening white globule with two tails (Fig. 8). Mature sporocysts freshly dissected from the mantle cavity do not show the swollen appearance, but the change takes place very soon, probably due to the atmospheric humidity or air pressure. The fluid inside the sporocyst begins to stream toward the anterior part of the body, evidently being forced into this part of the body by the rhythmic contraction of the outer cyst wall. At the same time, the membranous endocyst withdraws itself to the anterior part and coils up as the swollen portion enlarges (Fig. 7). The whole process takes about 30 minutes to complete. When the swollen cyst is formed, the outer cyst wall apparently dies, as no further movement is noticed. It becomes evident that such characteristic behavior of the daughter sporocyst is to provide a protection for the cercariae, which are now exposed to the external environment. Sporocysts moistened with saline solution and kept for more than 48 hours still contained living cercariae.

The cercaria: The cercaria is of a microcercous type. It is elongate-oval in shape and flattened dorso-ventrally. It measures from 0.33 mm to 0.37 mm in length and 0.112 mm to 0.140 mm in the greatest width (Fig. 9). The cuticle is aspinose except at the anterior and posterior regions of the body where it is armed with very minute spines. The small stumpy tail is often broader than long, measuring from 27 to 33 μ in length and 34 to 42 μ in width. On the posterior end of the tail there are some small spines resembling those on the tail of the microcercous cercaria of *Paragonimus*. The tail contains from 12 to 14 round nuclei, which can be seen clearly in the living condition. The oral opening of the cercaria is directed anterio-ventrad. It is surounded by a muscular oral sucker measuring 0.049–0.055 mm in diameter. A small stylet is located in a small cavity in front of the dorsal wall of the oral sucker with its point directed anteriorly. The acetabulum, which is situated on the middle portion of the body, measures 0.05–0.06 mm in diameter.

Fig. 6. A portion of daughter sporocyst under high magnification, showing body wall and two cercarial embryos.

Fig. 7. Mature daughter sporocysts in the process of contracting and forming the cyst-like structures.

The prepharynx is extremely short. The pharynx is globular, measuring 0.014-0.020 mm in diameter. The long, slender esophagus bifurcates in front of the acetabulum. Four pairs of penetration glands occupy the median region, adjacent to the long esophagus. Their ducts are usually combined into two bundles. Passing anteriorly in a sinuous course they open into the small pocket containing the stylet.



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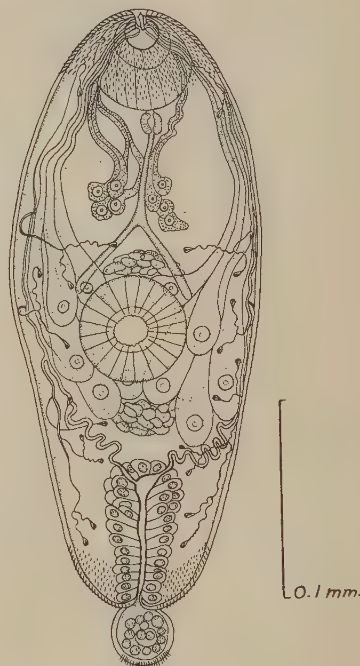

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FIG. 8. An expelled daughter sporocyst under high magnification.
FIG. 9. Cercaria of *E. pancreaticum.*

Five pairs of lateral penetration glands occupy space on either side of the acetabulum. The ducts of the lateral glands extend anteriorly in two large bundles, one on each side along the lateral margins of the worm. They also open into the small stylet pocket anterior to the oral sucker. The granules in these two types of penetration glands are different, being coarser in the median glands. The excretory bladder is tubular in shape. It is surrounded by a thick layer of elongate cells. On each an-

tero-lateral aspect the bladder receives a main collecting tubule which divides into an anterior and a posterior branch. The anterior branch proceeds anteriorly, the posterior one posteriorly, with each giving rise successively to three tubules: each of these tubules branches again into two capillaries ending with flame cells. The flame cell pattern is of the $2 \times 6 \times 2$ type.

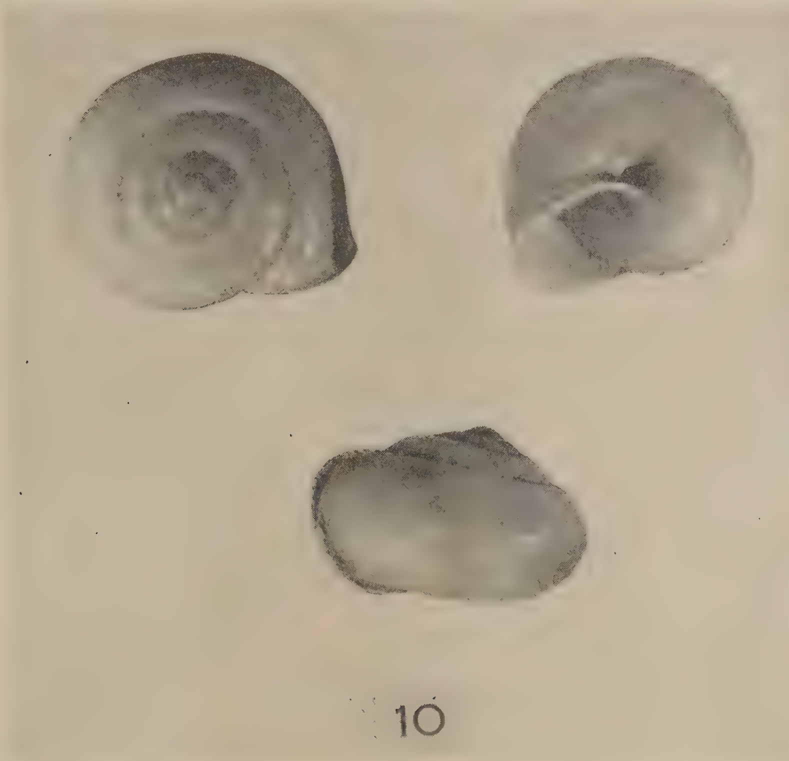

Fig. 10. *Bradybaena similaris* Férussac, intermediate host of *E. pancreaticum* in China.

DISCUSSION*

Looss (1907) erected the genus *Eurytrema* with *E. pancreaticum* Janson, 1889, as type. Bhalerao (1936) reviewed the literature on the genus and recognized seventeen species which he grouped into five subgenera. Travassos (1944) contributed an extensive monograph on the revision of the family Dicrocoeliidae Odhner, 1910, reducing the number of species in the genus *Eurytrema* to nine. These were divided into four subgenera.

Considerable divergence of opinion has existed as to the identity of *E. pancreaticum* and *E. coelomaticum* (Giard and Billet, 1892). The question arises as to whether these two are different species or only represent individual variations of

* This discussion except for the first two paragraphs was written by W. W. Cort. The author was forced to return to China before the final revision of this paper was made and had no chance to read the final copy of the manuscript or to correct the proof.

one. Looss believed that *E. coelomaticum* differs from *E. pancreaticum* in the relative size of suckers, while Travassos (1918) did not recognize *E. coelomaticum* as a distinct species. However, he listed it as such in his more recent revision of the family (Travassos 1944). Ware (1935) in his study of *E. pancreaticum* and *P. fastosum* showed great variation in individuals of these species. Bhalerao (1936) indicated the same for *E. pancreaticum* in his Indian material. According to the opinion of some authors (Bhalerao 1936, Yamaguti 1933), *E. ovis* Tubangui, 1925 and *E. satoi* Kobayashi, 1915 are to be regarded as synonyms of *E. pancreaticum.*

Including *E. pancreaticum* in the present study, the life cycles are now known, more or less completely, for five trematodes belonging to four different genera of the subfamily Dicrocoeliinae, viz., *D. dendriticum* (Mattes, 1933, 1936; Neuhaus, 1936, 1938), *E. procyonis* (Denton, 1944), *B. americanum* (Denton, 1945), and *P. fastosum* (Maldonado, 1945). The life cycles of these five species have certain resemblances to those of the superfamily Plagiorchioidea, but exhibit peculiarities which can be interpreted as adaptions to transmission by land snails.

The miracidia of all these five species are very similar in structure. They all hatch after the eggs are ingested by the snail host, and migrate into the digestive gland, where the mother sporocyst develops in the spaces between the lobes. In all five the mother sporocysts develop into very irregular sacs in close contact with the tissues of the snail. In them there is a very extensive multiplication of the small number of germinal cells that are brought over by the miracidium. In none of the dicrocoeliid species is the method of development of the germinal material brought out clearly. There is, however, aside from a great multiplication of the germinal cells in the mother sporocyst some suggestion of the presence of a germinal mass in the daughter sporocyst similar to those described for the plagiorchiids (Cort and Olivier, 1943; Cort and Ameel, 1944). Mattes (1936) stated that at the rounded posterior ends of the daughter sporocysts of *D. dendriticum* the wall was somewhat thickened and contained a cell mass capable of division. He considered this to be a kind of ovary. Neuhaus (1936, Fig. 12) described the wall of the daughter sporocyst of this same species as thin and lined with a flattened or cuboidal epithelium except at the posterior end where it consists of a "germ layer" or "mass." In a figure of a section of the posterior end of a daughter sporocyst he showed this "germ mass." It appears to be attached to the wall but is not really a part of it. His figure shows that this mass is composed of unicellular and multicellular components. He stated that the stage at which the embryo breaks away from the "germ mass" is not clearly defined because both germinal cells and germ balls were found free in the body cavity of daughter sporocysts. Also, Denton (1945) in his description of the daughter sporocysts of *B. americanum,* which are very similar to those of *D. dendriticum,* stated that "the group of small germ balls and cells, usually located at the posterior end of the cavity, is believed to be a germ mass from which additional cercarial embryos are formed." These descriptions suggest that in these two dicrocoeliids at least, there is a germinal mass in the daughter sporocysts like that of the plagiorchiids. If this mass is really definitely attached at the posterior end of the body cavity as suggested by Neuhaus' and Denton's descriptions, it would be a transitional type between the germinal masses of the rediae of the Fasciolatoidea (Cort, Ameel, and Van der Woude, 1948) and those of the daughter sporocysts of the plagiorchiids (Cort and Olivier, 1943; Cort and Ameel, 1944).

Perhaps the most interesting adaptations found in the life cycles of these five dicrocoeliid species are those that have developed for the transfer of the cercariae from the intermediate to the definitive hosts. The structure of the cercariae in the Dicrocoeliinae indicates clearly that they are related to the plagiorchiids, and most recent authors have recognized this relationship in their classification (cf. Faust, 1949, pp. 91 and 201). In the plagiorchiids, however, the larval stages are in water snails and the cercariae swim freely in the water and penetrate into second intermediate hosts. In *D. dendriticum* the cercariae, although retaining a long tail, have no free swimming stage but escape from the snail in large composite cysts which usually adhere to grass or other vegetation. The walls of both the individual and composite cysts appear to be formed from the secretions of the very large outer glands which fill most of the body of the cercaria. Infection of the definitive host is either by ingestion of the composite cysts on vegetation or by accidental ingestion of the snail intermediate host. Denton (1945) has observed a similar expulsion from the snail intermediate host of cercarial masses for *B. americanum,* a dicrocoeliid of passerine birds. For this species, however, he presented evidence that an insect second intermediate host is involved in the life cycle. In *B. americanum,* like *D. dendriticum,* the daughter sporocyst has a birth pore from which the cercariae escape and large glands are present filling most of the body of the cercaria. They also have a large tail which, however, is not adapted for swimming.

In the other three species of dicrocoeliids for which the life cycle is known the cercariae never leave the daughter sporocysts. They have a very much reduced tail and their outer glands are not highly developed, apparently because composite cysts are never formed. The daughter sporocysts of these three species have very thick walls and escape from the snail intermediate host. Denton (1944) stated that the mature daughter sporocysts of *E. procyonis* ruptured into the mantle cavity of the snail and collected in clumps, which were expelled from the respiratory pore by the movements of the snail and were deposited on vegetation or other subjects. In this case the daughter sporocysts are elongate oval structures with a very thick protective wall. The author was unable to determine the mode of infection of the definitive host or whether or not a second intermediate host was required. For *P. fastosum,* a dicrocoeliid of the cat from Puerto Rico, Maldonado (1945) has described a cercaria similar to those of the species of *Eurytrema.* In this case also the mature daughter sporocyst which is a very thick-walled structure escapes from the snail intermediate host. Although actual infection experiments have not been successful it is evident that the second intermediate host which is a lizard must be infected by ingesting these sporocysts. Infection experiments have demonstrated the transfer of the infection from lizards to cats and other animals. For *E. pancreaticum* the structure of the daughter sporocysts is more complicated than in the last two species discussed, but they also are expelled from the snail. Although it would seem possible that cattle and other herbivorous animals would get the infection by ingesting the daughter sporocysts with grass or other vegetation, attempts to produce infections by feeding the mature daughter sporocysts to goats have so far been unsuccessful. It is of course possible that in this species a second intermediate host is necessary.

The Dicrocoeliinae are a very numerous and wide-spread group. The five life cycles now known raise many interesting problems and indicate that further

studies in this group would be very profitable. We need to know much more about the development of the mother and daughter sporocysts, and of the various methods by which the cercariae reach the definitive host.

SUMMARY

The development in the intermediate host was worked out in experimental infections for *Eurytrema pancreaticum* Janson, 1889, a dicrocoeliid trematode which is common in the pancreatic and biliary passages of cattle and other herbivorous mammals in the Orient. The intermediate hosts that were demonstrated by the experimental infections are two land snails of the family Fruticoidolidae, *Bradybaena similaris* Férussac and *Cathaica ravida sieboldtiana* Pfeiffer, which were also found to be the natural intermediate hosts in Fukien Province, China.

The structure of the egg and miracidium of this species was found to be very similar to these stages described for several other Dicrocoeliinae. The eggs hatch only after being eaten by the snails, and the mother sporocyst develops in the tissue or the follicle of the digestive gland, closely surrounded by the host's tissue. The mother sporocyst becomes a large, rather irregular lobed structure. In its early development there is an extensive multiplication of germinal cells like that described for *Dicrocoelium dendriticum* and certain plagiorchiids. The daughter sporocysts develop, within the matrix of the mother, into large complicated sacs with a heavy outer wall which escape from the snails. The cercaria has a short stumpy tail and the structure of its body is very similar to that of the other dicrocoeliid cercariae that have been described. Since attempts to infect goats with the free daughter sporocysts were not successful it is not known whether or not a second intermediate host is required. While the structure of the larval stages of the Dicrocoeliinae show that they are closely related to the Plagiorchiidae, their life cycles show a number of striking differences from those of the latter group which are evidently modifications for transmission by land snails.

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EURYTREMA BRUMPTI RAILLIET, HENRY AND JOYEUX, 1912 (TREMATODA: DICROCOELIIDAE), FROM THE PANCREAS AND LIVER OF AFRICAN ANTHROPOID APES*

Horace W. Stunkard and Leonard J. Goss

Railliet, Henry and Joyeux (1912) described *Eurytrema brumpti* from some 300 specimens found in the hepatic and pancreatic ducts of an adult female chimpanzee which died in Paris. The animal came from the "Congo", but the precise location was not stated.

A massive infection with what is apparently the same species was discovered in a young male *Gorilla gorilla gorilla* (Savage and Wyman) which died in New York. At death the animal weighed 30.8 pounds and was probably about two years old. It had been brought by airplane from Africa on February 7, 1949, and although it came from the Congo region the locality where it was taken is uncertain. The animal was ill on arrival and shortly thereafter one of us, (L. J. G.) veterinarian of the New York Zoological Society, was called for medical advice and treatment. Its temperature fluctuated between 99 and 102 degrees F.; there were recurrent intestinal disturbances, anorexia, intermittent diarrhea and constipation. At times there were periods of two to four days in which there were no bowel movements. The fingernails had transverse bands of roughened areas, suggesting previous nutritional deficiencies or febrile conditions. Neither anemia or jaundice was noted.

One eyelid was injected with one-tenth cc. of 1 percent P.P.D. tuberculin; there was an immediate reaction. An edematous swelling of the lid completely closed the eye in a few minutes, but totally subsided within five hours. No tubercular lesions were observed at autopsy and the significance of the reaction is obscure. Several fecal examinations were made; hookworm eggs were present in large numbers but no trematode eggs were observed. To remove the hookworms, two treatments with tetrachlorethylene were administered but ova persisted in the stools. After the second treatment, diarrhea developed and the mucus contained large numbers of amoebae. Administration of carbarsone had no apparent effect on the amoebae; the dysentery continued and emetine hydrochloride was given. This treatment also proved ineffective; the stools became bloody and were unformed during the subsequent life of the animal. It failed to give the expected response to medication or to symptomatic and supportive measures. During a six week period it lost about four pounds and died of terminal pneumonia on April 2, 1949.

Autopsy of the gorilla was begun within three hours after death. The carcass showed no gross lesions other than early stages of lobar pneumonia, but the hepatic and especially the pancreatic ducts were filled with enormous numbers of small trematodes. Examination of the rectal contents revealed a few trematode eggs, but the number was not at all commensurate with the intensity of the infection. A piece of pancreas about 10 by 10 by 15 mm. was teased to bits in a Petri dish containing Ringer's solution. The worms were fixed and counted; 1028 specimens were present. Pieces of the liver and pancreas were removed, fixed and cut in serial

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sections, 10 microns thick. Some of them were stained with haematoxylin and erythrosin; others with the azan technique. Microscopic examination of the tissues showed cystic enlargements of the ducts (Plate II, fig. 1), erosion of the epithelium (Plate I, figs. 4, 5; Plate II, fig. 4) and pressure atrophy of the fundamental tissues. As a result of probably both mechanical and toxic irritation, there was invasion by leucocytes, increase in fibroblasts and hypertrophy of connective tissue. In places there was hypertrophy also of the epithelium and the formation of small adenomata. Lesions of duct walls had permitted eggs of the parasite and detritic material to pass into the tissue of the glands (Plate II, fig. 2).

A preliminary report of the findings was made by Stunkard (1949). Although the worms from the gorilla were slightly smaller than those described by Railliet, Henry and Joyeux, they manifested otherwise such precise agreement with *E. brumpti* that they were referred to that species. The slightly smaller size may be explained as a "crowding effect" from the intense infection and perhaps also as a result of development in a smaller host.

The food habits of the chimpanzee and gorilla are similar to those of the native tribesmen of the Congo basin. It would not be surprising therefore to find that *E. brumpti* is a parasite of man in that area. The probability is increased by the lack of precise host- specificity in *Platynosomum fastosum,* a closely related species which according to Mönnig (1947) is identical with *E. concinnum,* and the only one in which the life history is known.

All of the worms were sexually mature, but in many of them the ovary was small and the uterus almost entirely empty. The specimens varied greatly in shape (Plate I, figs. 1, 2, 3) and measured from 2.2–3.72 mm. in length and 1.3–2.1 mm. in width. The specimen shown in Plate I, fig. 1 measured 3.66 by 1.85 mm.; the preacetabular portion is well extended. The worms are thickest in the median portion and may measure 0.2–0.4 mm. at the level of the testes. The body becomes thinner toward the edges which are so delicate that they are often slightly wavy. The acetabulum is situated about one-third the body length from the anterior end but the preacetabular portion of the body, being more mobile than the posterior region which is filled with uterine coils, may be extended and thus shift the acetabulum relatively more posteriad. The acetabulum measures 0.25–0.30 mm. in diameter; the size depends on the degree of flattening.

The cuticula may be granular or covered externally with minute droplets, which give it a pebbled appearance. These droplets when compressed may appear as fine, closely spaced rugae, which suggest scales or spines; often the cuticula is faintly striated and frayed, but actual scales or spines have not been observed. Ducts from unicellular dermal glands are very numerous and the contents, which are refractile, may simulate the pattern of cuticular spines. The dermomuscular wall is thin and delicate; the circular, longitudinal and oblique fibers are not disposed in distinct layers. The body parenchyma is very loose and vacuolate, which renders the internal organs readily visible.

The mouth opening is ventral; the oral sucker subterminal, 0.25–0.29 mm. in diameter, approximately the same size as the acetabulum. The sucker is followed immediately by the pharynx, spherical to oval, and 0.09–0.126 mm. in diameter. The esophagus varies from 0.015–0.085 mm. in diameter and its length varies with the extension of the anterior end of the body. The bifurcation of the digestive tract is about one-fifth of the body length from the anterior end and a short distance posterior to the level of the genital pore. The ceca diverge at an angle, bend mediad and dorsad at the level of the vitellaria and then turn laterad, ending blindly in the areas behind the vitelline follicles. The walls of the ceca are thin and the lumen contains fluid in which fragments of cellular debris are sometimes recognizable. Apparently the worms are nourished almost entirely by fluid, sucked from the tissues of the host. The suckers, both oral and acetabular, may be attached to the walls of the pancreatic ducts (Plate I, figs. 4, 5; Plate II, figs. 3, 4) and often portions of the walls grasped by the oral sucker are held so firmly that the integrity of the tissue is broken (Plate I, fig. 5) and much extravasation must occur.

The excretory pore is terminal; the vesicle extends forward on the dorsal side of the body, above the uterine coils, about four-fifths of the distance to the oötype. Here it divides and the resulting ducts pass laterad and anteriad, mediad of the vitellaria, between these glands and the

gonads. The ducts turn ventrad, pass below the digestive ceca at the anterior border of the vitellaria and in the extracecal areas they divide into anterior and posterior branches which extend almost to the anterior and posterior ends of the body respectively. The capillaries and flame cells were not seen.

The testes are located on opposite sides, just in front of the middle of the body. Usually they extend forward to about the middle of the acetabular zone. They are irregularly notched or lobed, usually slightly longer in the anteroposterior dimension and 0.28–0.48 mm. in diameter. Vasa deferentia arise near the anteromedian faces of the testes and pass forward and mediad, joining as they enter the posterior end of the cirrus sac. The cirrus sac is 0.4–0.5 mm. in length and 0.125–0.18 mm. in diameter. It is a slightly curved structure and the posterior end is on the ovarian side of the body. Depending on the degree of extension of the anterior end of the body, the cirrus sac may be entirely preacetabular or it may extend to the middle of the acetabular zone. The posterior half of the cirrus sac is filled by a coiled seminal vesicle; the anterior half contains a coiled ejaculatory duct, surrounded by secretory cells. The cirrus is protrusible; in one specimen it is 0.22 mm. long, 0.045 mm. in diameter, with a terminal enlargement 0.07 mm. in diameter. The genital pore is median, ventral, slightly anterior to the level of the bifurcation of the digestive tract.

The ovary is notched or lobed, oval to irregular in shape, 0.12–0.20 mm. in diameter, usually longer in the transverse dimension, and situated a short distance behind one of the testes. It may be on either the right or left side of the body. The oviduct arises at the median, posterior margin and soon expands into a small seminal receptacle from which Laurer's canal passes mediad and dorsad, opening on the dorsal surface of the body near the anteromedian margin of Mehlis' gland. From the seminal receptacle a short duct leads to the oötype, which is surrounded by the cells of Mehlis' gland. Mehlis' gland is about as large as the ovary and situated posterior and median to it, but on the ovarian side of the body. The vitellaria are situated laterally in the ovarian zone in areas about the same size as the testes. Each vitelline gland consists of 10–16 follicles; the median follicles are ventral to the ceca. Vitelline ducts, filled with single rows of cells, pass mediad and dorsad; they unite at the dorsoposterior aspect of Mehlis' gland and form a vitelline receptacle which extends anteriad; a short duct passes from it ventrad to join the female duct just before it opens into the oötype. From the oötype the initial coil of the uterus passes to the ovarian side of the body and then across to the opposite side. The posterior half of the body is filled with uterine coils, 16–20 in number, which run transversely. In the proximal portion of the uterus the eggs have thin, colorless to yellow shells but the coils of the more distal portions contain darker colored eggs. The uterine coils extend forward between the testes and pass dorsally, where they open into the metraterm. The metraterm, about 0.06 mm. in diameter, with thick muscular wall, is about one-half the length of the cirrus sac and lies dorsal to it. The metraterm opens immediately in front of the cirrus at the common genital pore. Eggs in the initial portion of the uterus are small; those in the terminal portion have an average measurement of 0.45 by 0.27 mm. The mature eggs are operculate, oval to ovate, often slightly flattened on one side and contain fully formed miracidia.

The question of whether cuticular scales are present on *E. brumpti* is still unanswered. Looss (1907a) erected the genus *Eurytrema* with *E. pancreaticum* (Janson) as type and stated that in this species the cuticula is unarmed. This opinion was confirmed in another paper, Looss (1907b), in which he proposed a new but unnamed genus for *Dicrocoelium concinnum* Braun, 1901. He stated, "Ein Charakter ganz eigener Art, in dem *Dicroc. concinnum* von allen zur Zeit bekannten Dicrocöliiden abweicht, ist der Besitz eines feinen, dichten Schuppenkleides. Da aus anderen Beispielen bereits bekannt ist, dass die Ausstattung der Haut selbst bei nahe verwandten Formen verschieden sein kann, so dürfte auch in dem gegenwärtigen Falle die Bestachelung der Haut höchstens als Gattungscharakter betrachtet werden können. Denn Repräsentant einer eigenen Gattung ist *Dicroc. concinnum* ohne Zweifel, und diese Gattung stellt ein Bindeglied zwischen *Dicrocoelium-Platynosomum* und *Eurytrema* dar." In the original description of *E. brumpti,* Railliet, Henry and Joyeux reported, p. 834, "La cuticule est parsemée de très fines écailles, qui passent facilement inaperçues si on ne les recherche pas systématiquement." Further, p. 836 they stated, "On voit, par l'examen de ce

tableau, que nous faisons rentrer le *Dicrocoelium concinnum* Braun, 1901, dans le genre *Eurytrema,* bien que Looss, se basant surtout sur le présence de petites épines cutanées, tende à en faire le type d'un groupe intermédiaire entre les genres *Dicrocoelium* et *Platynosomum* d'une part, et *Eurytrema* d'autre part. Un examen minutieux nous a permis, en effet, de constater que ce revêtement épineux existe aussi chez les *Eurytrema* des Bovidés." Bhalerao (1924) described "small scales 20 μ to 50 μ, very thinly distributed and absent from the edges" of *E. dajii* n. sp., from the bile ducts of *Bos indicus.* In his (1936) description of specimens identified as *E. pancreaticum* Bhalerao stated, "The cuticula is smooth in all cases." Mönnig (1947) reported that *Eurytrema pancreaticum* bears cuticular spines, "which are often lost in the adult stage." As noted in the description of the specimens from the gorilla, no cuticular spines or scales could be found and a specimen labelled *Eurytrema coelomaticum* from the Looss' collection likewise is devoid of spines. Since the specimens from the gorilla were fixed while alive and active, there is slight possibility that spines or scales were lost before fixation.

The morphological and biological limits of the genus *Eurytrema,* and its relation to other genera in the subfamily DICROCOELIINAE, have been a problem since its erection by Looss (1907a). In a second contribution, Looss (1907b) erected a new genus *Platynosomum* and suggested other genera to contain related species, e.g., *Dicrocoelium concinnum.* A further difficulty concerns the extent of variation within single species and the features which distinguish between species within the genus *Eurytrema.* A summary of the taxonomic problems was made by Stunkard (1947) when specimens from the red fox, *Vulpes fulva,* taken near New York, were described and provisionally named as a new species, *E. vulpis.* Subsequently in correspondence, Dr. Denton stated that certain specimens of *E. procyonis,* described by him (1942) from the racoon, were very similar to *E. vulpis provis.* and suggested that the two were specifically identical. He sent a large number of specimens of *E. procyonis* for comparison and study of these worms has shown that there are no constant specific features which distinguish *E. vulpis* from *E. procyonis.* Consequently *E. vulpis* is suppressed as a synonym of *E. procyonis.*

In the paper cited, Stunkard (1947) discussed the subdivisions of *Eurytrema* proposed by Bhalerao (1936) and Travassos (1944). The characters selected to distinguish the five subgenera proposed by Bhalerao were regarded as inadequate and the same criticism applies even more strongly to the classification proposed by Travassos. The species included in *Eurytrema* manifest great morphological variations within a generalized generic pattern and, as noted by Denton (1944b), there appears to be no single character or set of characters on which the genus can be satisfactorily subdivided. The most plausible proposal yet made is that of Bhalerao. He included *E. brumpti* in the subgenus *Concinnum* which was raised to generic rank by Travassos. *E. brumpti* agrees with the characters listed by Bhalerao as diagnostic of the subgenus *Concinnum* although according to Mönnig (1947) *Platynosomum concinnum* (syn. *P. fastosum*) does not have transverse uterine coils anterior to the acetabulum. On the other hand, *E. alveyi,* a new species described by Martin and Gee (1949), contravenes the arrangement proposed by Bhalerao. In *E. alveyi* the ovary is larger than the testes which would place it in the subgenus *Skrjabinus,* but the genital pore is anterior to the bifurcation of the alimentary tract. In the latter feature and in the location of acetabulum and testes, it agrees with the

subgenus *Lubens*. In location of genital pore and extent of vitellaria, it might be assigned to the subgenus *Conspicuum*, but the extent of the uterus would exclude it from that group. The species does not conform to the diagnosis of any of the five subgenera and shows at least one character diagnostic of each of them. It is obviously improper to erect a new genus for each of the several variants, when all of them manifest variations around a common morphological archetype.

The bionomic features of the species of *Eurytrema* suggest that it is not a homogeneous group. The hosts include carnivorous and herbivorous mammals as well as rapacious, fructivorous and granivorous birds. The wide distributional range is undoubtedly correlated with the life cycles of the parasites and the food habits of their hosts. Unfortunately only a single life cycle has been discovered, that of *Eurytrema concinnum* (Braun, 1901) (= *Platynosomum fastosum* Kossack, 1910) by Maldonado (1945, 1946). The asexual stages develop in land snails, *Subulina octona*, an intermediate stage occurs in the bile ducts of lizards, and the adult stage normally develops in the livers of cats. In addition, Maldonado obtained mature worms from the experimental infection of white mice. Denton (1944a) described the asexual stages of *E. procyonis* in *Mesodon thyroidus* and the similarity of these developmental stages to those of *E. concinnum* and the location of the adult stages in racoons and foxes, suggests a life history like that of *E. concinnum*. But it is hard to believe that the species of *Eurytrema* from small, seed-eating birds have a developmental stage in the bile ducts of lizards. Denton (1945) described the asexual stages of *Brachylecithum americanum*, a related dicrocoelid species from the livers of various passerine birds, and compared them with corresponding stages of *Dicrocoelium dendriticum* and *Eurytrema procyonis*. Evidence was presented that the cercariae enter larvae of chrysomelid beetles which serve as second intermediate hosts. If eurytremid parasites of birds employ insects as intermediate hosts, bionomic characters may provide a possible basis for specific and generic determinations. When the life cycles of avian species are discovered, it should be possible to correlate bionomic and morphological data with taxonomic considerations and permit a proper disposition of the species of *Eurytrema*.

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Plate I

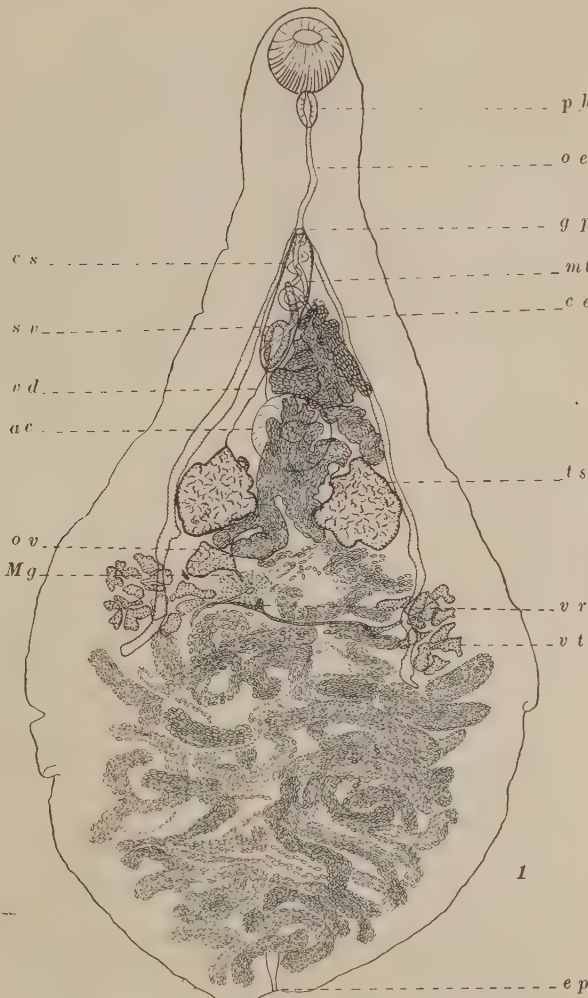
Fig. 1. Whole mount of *E. brumpti;* a well extended specimen.

Figs. 2, 3. Outline drawings of other specimens of *E. brumpti*, to illustrate variations in shape of body and position of the gonads.

Fig. 4. Section of pancreas with acetabular sucker attached to the wall of a tubule.

Fig. 5. Section of pancreas with oral sucker attached to wall of tubule. Note absence of epithelium and derangement of subepithelial tissues.

PLATE I



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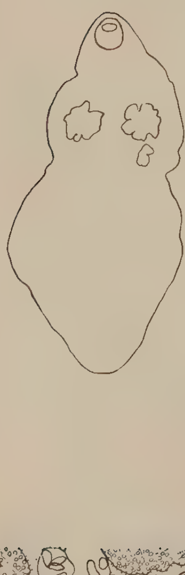

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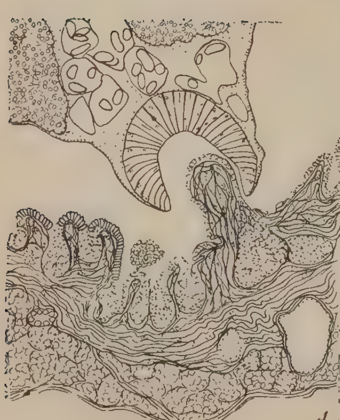

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Explanation of Figures

Abbreviations

ac	acetabulum	ov	ovary
ce	digestive cecum	ph	pharynx
cs	cirrus sac	sv	seminal vesicle
ep	excretory pore	ts	testis
gp	genital pore	vd	vas deferens
Mg	Mehlis' gland	vr	vitelline receptacle
mt	metraterm	vt	vitellaria
oe	esophagus		

Plate II

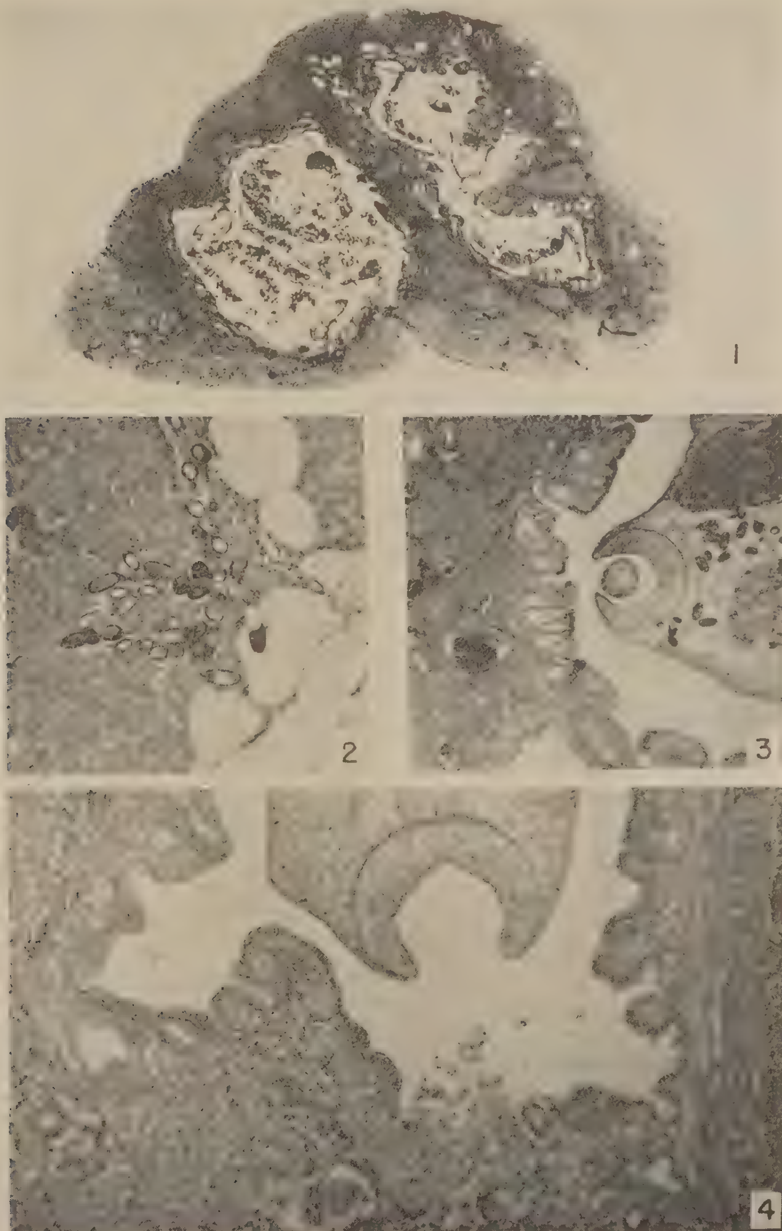
Fig. 1. Section of pancreas, low power, showing enormously enlarged ducts, filled with *E. brumpti*. The worm in the lower left portion of the photograph is the one shown on Plate I, fig. 5.

Fig. 2. Section of pancreas showing eggs in the tissue.

Fig. 3. Section of pancreas showing portion of duct wall in acetabular sucker of a worm; same specimen shown in Plate I, fig. 4.

Fig. 4. Section of pancreas, showing denuded area where a portion of the wall of a duct has been torn off.

PLATE II



SULFONAMIDE THERAPY OF TRICHINIZED WHITE MICE

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In an excellent review Van Someren (1939) presented a list of substances tried for the treatment of trichinosis. None of the substances listed were very satisfactory.

McCoy (1938) reported that daily doses of 125 mg. of sulfanilamide were ineffective for the treatment of trichonosis in rats. On the other hand a limited study by McNaught, et al (1939) indicated that sulfanilamide administered in the feed at a 1.6 percent concentration affected a larval reduction of 55.0 percent in rats. In view of this observation Riedel and Lunde (1949) undertook an extensive investigation of sulfanilamide and other sulfonamides of medicinal interest to learn their therapeutic possibilities for the treatment of trichinosis in white mice. Rations containing sulfaypridine, sulfathiazole or sulfadiazine at maximum nontoxic levels were ineffective; sulfaguanidine was indefinite; and a 2.0 percent sulfanilamide ration affected a larval reduction of 54.0 percent.

The objective of the present study was to compare the effects of sulfanilamide with sulfamerazine and sulfaquinoxaline upon trichinae, and to note if sulfonamide therapy affected a reduction of the mortality among white mice heavily infected with *Trichinella spiralis* larvae.

METHODS

The white mice used in this investigation were from a strain used in previous research (Riedel, 1948; 1949; 1950; and Riedel and Lunde, 1949). All infections, unless otherwise stated, were made by feeding with forceps small portions of freshly killed mouse diaphragm containing counted numbers of encysted larvae. The larvae harbored were recovered and counted by a method described by Riedel (1949). Age and sex were carefully considered when grouping the mice.

From the results of Riedel and Lunde (1949) and preliminary experimentation, it was found that the sulfonamides could be safely administered in the feed to mice at the following levels of concentration:

Sulfanilamide	2.0 %
Sulfamerazine	1.5 %
Sulfaquinoxaline	0.25%

In the feeds which contained combinations of sulfonamides, the sulfanilamide and sulfaquinoxaline were left at the 2.0 and 0.25 percent levels, respectively. The sulfamerazine content was reduced to 1.0 percent.

RESULTS

Table 1 shows two series each of control and sulfonamide-treated groups of mice. The mice were individually infected with 125 ± 5 *Trichinella spiralis* cysts. Treatment was begun immediately after infection and the mice were autopsied 30 days later.

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The data (Table 1) show that sulfanilamide affected an average larval reduction of 57.7 and 50.4 percent, respectively, among the animals in each of the series. The sulfaquinoxaline did not reduce the number of larvae harbored. The larval reduction among the sulfamerazine-treated mice was 52.4 percent in the first series, and 59.3 percent in the second series. The sulfonamide efficiency was the greatest among the mice given a combined treatment of sulfanilamide and sulfamerazine. The average larval reduction in the hosts was 67.1 percent in the first series, and 79.9 percent in the second series. A comparison of the numbers of larvae harbored by the sulfanilamide-sulfaquinoxaline treated groups of mice with those harbored by the sulfanilamide treated groups of hosts was further evidence of the ineffectivity of sulfaquinoxaline as a therapeutic agent.

Sulfanilamide and sulfamerazine were shown to reduce the numbers of *Trichinella* larvae harbored in mice, but this larval reduction did not indicate that the sulfona-

TABLE 1.—*Numbers of* T. spiralis *larvae harbored by each of the sulfonamide and control groups of mice*

	Control	Sulfanilamide	Sulfaquinoxaline	Sulfamerazine	Sulfanilamide and Sulfamerazine	Sulfanilamide and Sulfaquinoxaline
			Series 1			
	38140	13670	47150	9260	3650	11400
	16700	9700	22550	15460	8110	6330
	24420	14300	19070	13120	11400	4010
	40870	12950	18450	13940	9000	19090
	46000	10430	37940	6690	14230	17440
	19730	12390	43810	22890	6680	4500
	28660	15000	22180	14670	11020	6550
	23260	8020	10330	10100	13970	12980
	18190	9780	32790	16450	8540	13760
	21870	11120	21260	9880	4900	19110
Av.	27784	11736	27553	13246	9150	11517
			Series 2			
	9900	18430	27810	11260	7060	14440
	42990	6800	21140	9100	4430	13650
	26500	8460	37910	18600	9260	13310
	13340	14800	8340	12230	6700	14980
	31400	16900	41000	2360	5510	16340
	31890	9220	17910	16970	9780	8170
	19770	11470	19660	18580	14000	10010
	28800	13440	24040	3910	850	12120
	24110	14020	29600	176	9432	9480
Av.	25411	12616	25268	10332	7448	12500

mides would protect the host from severe, lethal infections of the parasite. This led to another experiment performed on several groups of mice. The experimental animals were individually infected with 1300 larvae. The larvae were obtained and injected into the stomach as described by Larsh and Kent (1949).

Treatment began immediately after infection and was discontinued 30 days later. The experiments were terminated on the 50th day. The sulfonamide dosage was the same as that of the mice represented in Table 1. The sulfaquinoxaline was omitted from this portion of the investigation.

The data in Table 2 show that one of the noninfected control mice died during the 50-day experimental period. The rate of survival among the nontreated, infected, control mice was 11.1 percent, and all of them showed symptoms of trichinosis at the end of the experiment. The sulfanilamide and sulfamerazine administered to mice permitted a survival of 62.2 and 71.1 percent, respectively. Among the mice

treated with a combination of sulfanilamide and sulfamerazine, 82.2 percent were still alive at the end of the experiment.

All the animals in the infected, control groups showed symptoms of the disease at some time during the 50-day, experimental period; and no recovery from the symptoms occurred. Among the mice treated with a combination of the sulfonamides, 11.1 percent remained without symptoms of trichinosis. For each of the sulfanilamide and sulfamerazine groups of hosts the rates of morbidity were 89.0 and 100.0 percent, respectively; but many of these animals showed signs of recovery from the disease before the 50th day.

With respect to toxicity, the sulfanilamide and sulfamerazine were well tolerated. The sulfaquinoxaline produced signs of toxicity by the 12th day. The mice fed the combination of sulfanilamide and sulfamerazine showed symptoms of toxicity on the 10th day of treatment. When symptoms of toxicity developed, the continuous method of treatment was abolished and the feed was administered on a 2 : 1 : 2 basis. Plain feed was administered on the single day. Post mortem examinations of all the

TABLE 2.—*Mortality of sulfonamide and control groups of mice infected with large numbers of* T. spiralis *larvae*

Number of Mice	Noninfected controls	Infected controls	Sulfanilamide	Sulfamerazine	Sulfanilamide and Sulfamerazine
		Trial 1			
At start	10	10	10	10	10
At finish	10	0	6	6	8
		Trial 2			
At start	15	15	15	15	15
At finish	14*	3	11	9	13
		Trial 3			
At start	20	20	20	20	20
At finish	20	2	13	13	16
		Totals			
At start	45	45	45	45	45
At finish	44	5	32	28	37

* One mouse died from an ulcerous sore.

mice which died during the experimental period indicated that the cause of death had been from trichinosis and crystallization of sulfonamides in the renal tubules was not apparent.

When the experiment was terminated the surviving mice were autopsied and grossly examined for the presence of cysts. All of the sulfonamide treated animals were infected.

DISCUSSION

In studies of age resistance in mice infected by *Trichinella,* Riedel (1950) found that among old mice, elimination of the adult parasite was rapid within 15 days after infection; but among young mice elimination occurred more slowly. For this reason treatment in the present investigation was not continued beyond 30 days after infection with the parasite.

In the present study, the mortality among the infected, control mice was heaviest from the 10th to the 30th day after infection. This corresponded with mortality records presented by Rappaport (1943). Among the treated mice the onset of the disease was retarded and the heaviest mortality occurred from the 16th to the 26th

day after infection. This indicated that sulfonamides tended to delay and shorten the period of mortality.

Sulfanilamide and sulfamerazine administered alone were not completely effective, and administering the drugs at higher than the nontoxic levels used in this investigation would have been hazardous. A combination of the two sulfonamides, each at about the maximum nontoxic level, resulted in increased drug efficiency without dangerously augmenting the toxic effects of the individual sulfonamides. In studies of human brucellosis Spink, et al, 1948) and bovine mastitis (Johnson and Roberts, 1947) it has been found that the efficiency of sulfonamides may be increased when administered in combination with other relatively ineffective antibiotics as streptomycin or penicillin.

SUMMARY

An investigation was performed on several groups of trichinized mice. The results showed that continuous feeding of 0.25 percent of sulfaquinoxaline did not effect a larval reduction. A 2.0 percent sulfanilamide feed effected a larval reduction of 57.7 and 50.4 percent, while 1.5 percent of sulfamerazine was 52.4 and 59.3 percent effective. Combinations of sulfanilamide and sulfamerazine reduced the larval counts 67.1 and 79.9 percent.

Among the control mice infected with a lethal dose of larvae, 11.1 percent survived the 50-day, experimental period. Sulfanilamide and sulfamerazine effected a survival of 62.1 and 71.1 percent, respectively. The rate of mortality was reduced to 17.8 percent by a combination of sulfanilamide and sulfamerazine. The period of mortality was distinctly shortened among the sulfonamide groups of mice.

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THE RELATIONSHIP OF POPULATION GROWTH TO *IN VITRO* ENCYSTATION OF *ENDAMOEBA HISTOLYTICA*[1]

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INTRODUCTION

Since Dobell and Laidlaw (1926) found that the addition of rice starch to a culture of *E. histolytica* resulted in encystation, many attempts have been made to determine the factors which are responsible for the process. The rôle of the associated bacteria has been discussed by several investigators (Cleveland and Sanders, 1930; Chinn *et al.*, 1942; Chang, 1942, 1946), all of whom were of the opinion that certain bacteria favor encystation. Chang (1942, 1946) made extensive studies concerning the influence of hydrogen-ion concentration and oxidation-reduction potential. He found that a pH value of 6.8 to 7.0 was most favorable and that conditions providing low potential followed by a sharp rise in potential induced mass encystation after a greatly increased rate of multiplication of trophozoites. This observation that encystation was preceded by abundant growth led to the present study, in which an attempt has been made to learn more concerning the effect of the factor of growth upon encystation.

MATERIALS AND METHODS

Two strains of *Endamoeba histolytica* were used in these experiments. Strain 22, obtained through the courtesy of Dr. J. C. Swartzwelder of Louisiana State Medical Center, was isolated from a human brain abscess. The bacterial flora of this strain, when first examined in the cultures, consisted of five Gram-negative rods of the *Alkaligenes* group, and a Gram-positive *Micrococcus* (Carrera and Faust, 1949). Strain 23, generously provided by Dr. Paul C. Beaver of our department, was cultured from an apparently symptomless case. The flora of this strain included a large Gram-positive bacillus and two small Gram-negative bacilli. With the exception of the first series of experiments, only strain 22 was employed because strain 23 was unfortunately lost in culture.

The routine procedure used for the production of cysts was based on the method of Dobell and Laidlaw (1926). Cultures were maintained in a starch-free medium, which consisted of a whole egg-slant with buffered saline overlay. To obtain cysts, subcultures were made into Balamuth's (1946) dehydrated egg-yolk infusion plus 0.5% liver extract (Lilly) to which especially ground rice powder had been added (obtained through the kindness of Dr. C. W. Rees, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Md.). An inoculum of known size was introduced into a measured quantity of Balamuth's medium in order to make a

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mensural study. The inoculum was obtained from the sediments of a number of starch-free cultures which were pooled, counted, and diluted or concentrated to the desired number of amebae per ml. The size of inoculum, quantity of medium, and the counting intervals varied according to the conditions of the experiment (to be described under the individual headings).

Counts were made of thoroughly mixed cultures using the Spencer bright-line hemocytometer, after the method of Paulson (1932). From six to ten counts were made at each determination, the average of nine squares being multiplied by 10,000 to obtain the number of trophozoites and cysts per ml., and the final population calculated by multiplying that product by the total volume of medium.

RESULTS

1. *Comparison of strains 22 and 23.* In the first series of ten experiments, inocula ranging from 100 to 50,000 amebae were introduced into 20 × 150 mm. culture tubes containing 14 ml. of Balamuth's medium plus about 30 mg. of rice powder. Counts at 24 hours revealed few or no cysts. At 72 hours there was a diminution in numbers of trophozoites and cysts in those tubes that received inocula of 5,000 amebae or more, while there was some increase in both where the inocula were less. Data collected at 48 hours seemed to indicate that this was the midpoint in the cycle and therefore the optimum time for comparison.

TABLE 1.—*Comparison of total population, rate of growth, and percentage encystation of two strains of* Endamoeba histolytica

Size of inoculum	Strain 22			Strain 23		
	Total population in thousands	Number div./day	Per cent cysts	Total population in thousands	Number div./day	Per cent cysts
50,000	930.0	2.3	9.9	2,120.0	3.1	37.3
10,000	765.0	3.5	11.5	2,300.0	4.4	39.7
5,000	810.0	4.3	11.3	2,420.0	5.1	41.5
1,000	21.0	2.6	0.0	515.0	5.1	5.6
500	6.0	2.0	0.0	210.0	5.0	3.1

Growth from inocula of 100 amebae was sporadic, observed in only a few instances, and was excluded from the tabulations. The data summarized in table 1 show that there was a significant difference in the degree of encystation of the two strains, particularly from inocula above 5,000 amebae ($P < .005$).[3] In an effort to account for the differences that were occurring, some observations made on the pH showed that the initial pH of the medium was 7.2 and that throughout the incubation period the range for both strains was 6.8 to 7.1 An attempt was also made to determine the rate of multiplication, as a means of explaining the results, since Chang (1942) had linked the process of encystation, at least in part, with an accelerated rate of growth. The calculation based on the method of Brackett and Bliznick (1947) could only be considered as an approximate means of comparison, since neither mortality nor rate of encystation could be taken into account. By this method the rate of growth is expressed as the number of binary divisions per day, and the differences between the two strains are shown in table 1. With strain 23, inocula of 1,000 and 500 amebae resulted in the same high rates of multiplication as

[3] Student's "t" test was used for all tests for significance.

larger inocula, although the differences in percentage of encystation were considerable. Probably these cultures continued to multiply, as indicated by the few 72-hour counts which were made, reaching the growth peak and maximum encystation at a later time. However, attention was soon drawn to the correlation between the size of the populations of the cultures and the percentage of encystation. Strain 23, with inocula above 5,000, reached populations above two million, with approximately 40 per cent cysts. Strain 22, with a similar amount of inoculum, reached populations of somewhat less than one million, with approximately 10 per cent cysts. Correspondingly small populations and few or no cysts were noted from inocula below 5,000 amebae.

2. *Further studies of growth and encystation of strain 22.* The earlier experiments suggested that more emphasis should be placed on size of population in relation to encystation.

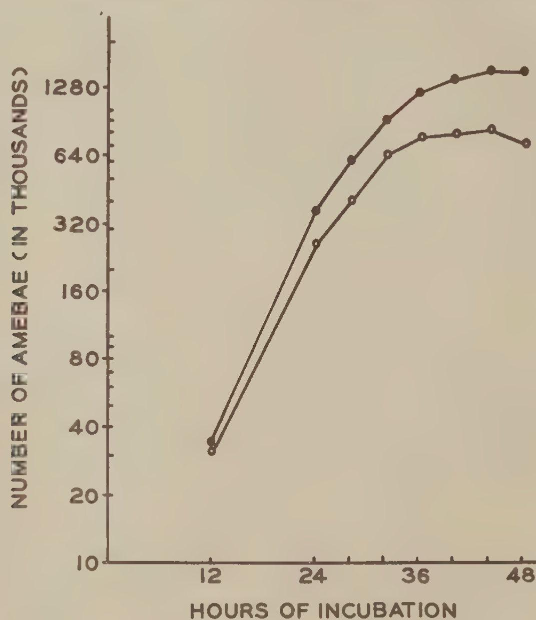

Fig. 1. Effect of stirring cultures on the growth cycle of *Endamoeba histolytica* (Strain 22). Each solid circle is the average of eight cultures stirred and counted at each of the indicated time intervals; each open circle is the average of eight cultures stirred and counted at a single time interval only.

Additional work was indicated, not only for support of the evidence already obtained but also to determine when encystation began and when the maximum was reached. In this series of experiments, an inoculum of 25,000 amebae in 1 ml. was used. Ten 20×150 mm. culture tubes containing 14 ml. of Balamuth's medium plus 30 mg. of rice powder were inoculated for each trial series. After 12, 24, 28, 32, 36, 40, and 44 hours of incubation, tube 1 (see table 2) was stirred, but no samples were removed for counting until 48 hours. The amebae of tube 2 were thoroughly distributed by stirring and samples were counted at each of the above time intervals. Those of tube 3 were stirred and counts were made at 12 and 48 hours; those of tube 4 at 24 and 48 hours; those of tube 5 at 28 and 48 hours;

and so on until those of tube 10 were left undisturbed until 48 hours when counts were made. Since the effect of frequent agitation on multiplication and encystation was not definitely known, it was considered necessary to use previously undisturbed cultures at each counting period as well as cultures which were only stirred or both stirred and counted each time. This procedure was carried out ten different times. In two experiments the amebae failed to grow appreciably in any of the tubes of the series. The average results for the eight remaining experiments are shown in table 2. The summary of results in table 2 shows: (1) That the cultures which were disturbed at each interval grew more rapidly and abundantly throughout the cycle than did the remaining cultures, although this difference was not statistically significant until 40 hours ($P < .05$); (2) that encystation in these cultures which were frequently disturbed was almost completely inhibited; (3) that encystation of the other cultures began at 28–32 hours and reached a peak at 44–48 hours, and (4) that

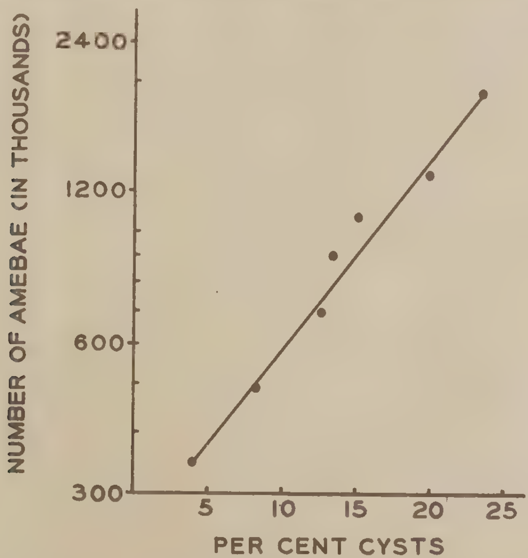

Fig. 2. Relationship of average per cent of cysts to average total population (plotted on logarithmic scale) of cultures of *Endamoeba histolytica* (Strain 22) at the time of maximum encystation. Based on averages from 138 cultures disturbed not more than once during the incubation period. (As an example, see table 2, vertical columns 3–10.)

agitation of a culture several hours after the onset of encystation prevented further encystation or caused a rapid decrease in the number of cysts.

Figure 1 is a graphic representation of the relationship between the growth cycle of the amebae in tubes which were stirred and counted at frequent intervals and those from single tubes stirred and counted at one of the given intervals only, as described above. The pattern, shown here on a logarithmic scale, is similar for both, although, as shown in table 2, encystation began and continued to mount in the single interval tubes as growth leveled off. In the agitated cultures, on the other hand, the amount of encystation remained negligible in spite of the rapid rate of multiplication, the size of the population, and the fact that growth began to level off at about 32 hours.

TABLE 2.—*Average number of* Endamoeba histolytica *(strain 22) and average per cent encysted in tubed cultures counted at varying times following initial inoculation with 25,000 amebae**

Hours of incubation	Average population in ten thousands and percentage of cysts (in parenthesis)†									
	1	2	3	4	5	6	7	8	9	10
12	stirred	3 ± .4 (0.0)	3 ± .4 (0.0)							
24	"	35 ± 8 (0.0)		25 ± 6 (0.0)						
28	"	60 ± 13 (.02 ± .02)			38 ± 10 (0.0)					
32	"	88 ± 15 (.05 ± .03)				63 ± 14 (1.2 ± 0.8)				
36	"	117 ± 18 (0.3 ± 0.2)					75 ± 17 (5.8 ± 2)			
40	"	134 ± 18 (0.7 ± 0.5)						76 ± 12 (10.8 ± 2.6)		
44	"	146 ± 16 (0.7 ± 0.5)							80 ± 11 (12.2 ± 2.5)	
48	166 ± 21 (0.8 ± 0.6)	145 ± 8 (0.7 ± 0.5)	75 ± 13 (11.0 ± 2.4)	82 ± 15 (12.7 ± 3.0)	102 ± 22 (13.8 ± 2.5)	108 ± 23 (11.7 ± 1.8)	96 ± 11 (11.6 ± 2.4)	94 ± 12 (6.7 ± 1.5)	83 ± 10 (4.8 ± .8)	69 ± 11 (11.1 ± 2.5)

* **Results are unweighted averages of eight individual experiments.**
† Shown with standard error.

The results shown in figure 2 suggest that the increase in percentage of cysts is directly proportional to the logarithmic increase in the size of the population. The points on the graph represent the average of a particular population level (for example, 200,000 to 400,000, 400,000 to 600,000, etc.) plotted against the average per cent of cysts for the same population level. The figures were based on a collection of results from 138 culture tubes (20×150 mm.) from various experiments at the time of maximum encystation—48 hours or somewhat earlier. All tubes contained the same amount of medium and had received the same size of inoculum (25,000 amebae). The cultures were disturbed only once or not at all before the final determination.

3. *Comparison of growth and encystation of strain 22 in containers of different sizes.* Since the size of the population and therefore the factor of "crowding," appeared to be playing a rôle, at least in part, in the process of encystation in cultures which were not disturbed, the use of containers of different sizes was considered as a means of further testing the possibility. In still another series of five experiments, one group of culture containers received inocula varying with the amount of medium on a per ml. basis, and in the second group, the inoculum was constant. In the first group, 25 ml. Erlenmeyer flasks (22 ml. medium), 16×120 mm. tubes (22 ml. medium), 18×150 mm. tubes (7 ml. medium), and 10×120 mm. tubes (2.64 ml. medium) all received inocula of approximately 3,000 amebae per ml. of culture medium, making a total of 75,000, 75,000, 25,000 and 9,000 amebae respectively. In the second group, the same containers, with 24 ml., 24 ml., 14 ml., and 4 ml. of medium respectively, received inocula of 25,000 amebae. The amount of rice powder used varied with the size of the containers. Counts were made at 24 and 48 hours.

The various sizes and shapes of the vessels made possible the counterbalancing of variable conditions against one another, such as the depth of the medium, the amount of medium, the surface area exposed to air, the bottom surface area of the container, the bacterial inoculum and the amebic inoculum. For example, using the symbols designated in table 3, conditions were similar in flasks A and tubes B except for the depth of medium and the surface available to multiplying amebae; flasks A and tubes C were equalized with the exception of the amount of medium and the surface available to the amebae. Flasks A_1 and A_2 had comparable conditions except for the size of inoculum, and so forth. The summary of results is shown in table 3. The amebae in the flasks multiplied at a rapid rate and attained much larger populations than those of the tubes, yet there was a lower percentage of encystation or none at all in the case of flask A_2. In comparing flask A_1 with flask A_2, there was a significant difference in the amount of growth ($P < .01$), while in comparing like tubes, despite the differences in inoculum, there were no essential differences in either growth or percentage of encystation. The amebae of tube D_2, however, began encystation earlier, and therefore probably reached a peak long before 48 hours. Even in the case of flask A_1 and tube B_1, where both inocula and amount of medium were similar, there was a vast difference in the final outcome. There is an indication that the amount of growth was considerably influenced by the surface available to the multiplying amebae, irrespective of the amount of medium, and to a considerable extent, also the size of inoculum. Likewise, the size of the population necessary to stimulate encystation appeared to depend on this same factor of area.

The close relationship that existed between the amount of growth and the bottom surface area is shown by means of the ratios presented in table 4. For example, the population of flask A_1 divided by that of tube B_1 gave a ratio of 7.3 : 1, and similarly the ratio of the areas was found to be 7.2 : 1. The area of various vessels was

TABLE 3.—*Growth and encystation of* Endamoeba histolytica, *strain 22, in containers of various sizes*†

Symbol for container and inoculum	Type of container	Ml. of medium	Ml. of inoculum	Number of amebae in thousands: At inoculation (0 hours)	24 hours: Total population	24 hours: Per cent cysts	48 hours: Total population	48 hours: Per cent cysts
A_1	Flask 25 ml.	22	3	75*	1,281	0.0	4,768	9.0
A_2	Flask 25 ml.	24	1	25	150	0.0	2,788	0.0
B_1	Tube 20 × 150 mm.	7	1	25*	443	0.0	1,060	13.0
B_2	Tube 20 × 150 mm.	14	1	25	265	0.0	1,018	15.2
C_1	Tube 16 × 150 mm.	22	3	75*	291	0.5	653	19.0
C_2	Tube 16 × 150 mm.	24	1	25	109	0.0	720	12.6
D_1	Tube 10 × 120 mm.	2.64	0.36	9*	127	0.0	293	14.5
D_2	Tube 10 × 120 mm.	4	1	25	159	3.3	260	6.3

† Based on five individual experiments, two of each container per trial.
* Approximately 3,000 per ml. of culture medium.

roughly calculated from the measurement of the diameter of the surface covered by rice powder.

DISCUSSION

The foregoing data have shown a close relationship between the density of population and encystation. While adding another element to the group of factors which possibly have influence upon the production of cysts, the results have raised the question concerning the nature of this relationship. It has been observed by Chang (1946) and during the present study that the trophozoites and cysts tend to be

TABLE 4.—*The relationship between bottom surface area and the amount of growth at 48 hours*

Type of container	Mean population in thousands	Bottom surface area in sq. mm.
Flask A_1 25 ml.	4,768	962
Tube B_1 20 × 150 mm.	1,060	201
Tube C_1 16 × 200 mm.	653	133
Tube D_1 10 × 120 mm.	293	64

Container combination	Population ratio	Area ratio
A_1/D_1	16.3	15.0
A_1/C_1	7.3	7.2
A_1/B_1	4.5	4.8
B_1/D_1	3.6	3.1
C_1/D_1	2.2	2.1
B_1/C_1	1.6	1.5

found in clumps. Balamuth and Howard (1946) noted that stirring a culture gave rise to increased growth, which they thought possibly was due to the breaking up of the clumps of amebae so that more effective use could be made of the bottom surface of the tube. We have seen in the present study that frequent agitation of a culture,

while apparently causing more rapid growth, resulted in little or no encystation of the amebae. Furthermore, agitation of a culture, in which the process of encystation had been taking place for several hours, reduced the number of cysts and seemed to inhibit almost completely any further cyst formation. Such agitation, as was suggested above, would scatter the multiplying trophozoites so that there would not be a large concentration of the organisms for a prolonged period of time. It was also shown that in small tubes, cysts were produced in conjunction with small populations, while in flasks there is no encystation until a very large population has accumulated. These observations indicate the possibility that physical crowding may be influencing the amebae to encyst. However, under these conditions in which numerous amebae have been accumulating, there is also the possibility that some by-products of metabolism are being produced and when in sufficient concentration, induce encystation. This could be related to several of the observations as follows: (1) Encystation did not occur *en masse* but was a gradual process which took place throughout a 12- to 16-hour period; (2) frequent agitation of a culture or stirring after encystation was well under way would disperse any substances which had accumulated in the bottom of the tube; (3) the broad area of a flask would allow a greater opportunity for diffusion of substances throughout the medium. The presence or absence of such substances which would induce cyst formation has yet to be demonstrated, but sufficient evidence has been obtained to indicate that further investigation is warranted.

Certain observations concerning growth and cyst production in strain 22 deserve brief mention. The trend shown in figure 1 was relatively constant throughout the series of experiments, although there was considerable variation in absolute numbers despite the fact that conditions appeared to be the same for each experiment. This has been the major difficulty encountered by investigators of the phenomenon of encystation of *E. histolytica.* Some attempts were made to relate the observed differences to the age of the encystation medium and to the different lots of media, but the findings were inconclusive. The dilution of the inoculum with reference to the bacterial flora was also considered, since the amount of diluent (Balamuth's medium) used to obtain the desired number of trophozoites per ml. was not always the same. Also, it was sometimes necessary to concentrate the organisms by means of centrifugalization. Better growth generally appeared to follow the use of an inoculum in which the pooled sediments of the starch-free cultures were diluted by approximately half, but this was not necessarily the rule and the evidence could not be considered conclusive. In a few trials, the use of media preconditioned with bacteria failed to throw any further light on the subject. Although growth was initially more rapid, the amount of growth and encystation was not essentially different from that of un-preconditioned cultures at the 48-hour determination. It was observed that in each individual experiment, in which a group of tubes had received inocula from the same pool at the same time, the results were consistent. Tentatively it may be concluded that the results obtained depend on chance inoculation at a time when either the amebae or the bacterial associates, or both, are at a particular stage of development which predisposes to optimum conditions for growth and subsequent encystation. It has also been noted that the cysts produced *in vitro* by this strain were invariably uninucleate, regardless of the time of observation. They were, however, capable of excystation. This property was tested following treatment with

HCl and/or refrigeration to insure that no trophozoites were present. It remains to be demonstrated whether the production of uninucleate cysts is inherent in strain 22, or merely due to the conditions of the method used for encystation.

SUMMARY

Cysts of *E. histolytica* were produced *in vitro* by transferring trophozoites from a starch-free medium into one containing starch. Two strains (nos. 22 and 23) were compared with regard to encystation, and the results indicated that the differences observed in the percentage of encystation were related to the density of population of the cultures. Further investigations with strain 22 revealed that the increase in percentage of cysts in essentially undisturbed cultures was directly proportional to the logarithmic increase in the size of the population. In a study of growth and encystation in vessels of various sizes, it was found that the population increased in proportion to the horizontal surface available to the amebae, and that the size of population necessary to stimulate encystation depended also on this area. These observations indicate a connection between the production of large numbers of amebae and the phenomenon of encystation, which may possibly be attributed to physical "crowding" of the organisms, or to the accumulation of metabolic by-products from the multiplying amebae.

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TWO NEW BLOOD FLUKES FROM A MARINE TURTLE, *CARETTA CARETTA**

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The trematodes described below were collected by the senior author from a loggerhead turtle, *Caretta caretta,* at the Biological Laboratory of the Carnegie Institution at Tortugas, Florida, in June, 1932. Seven other species of trematodes, including one other blood fluke, *Hapalotrema synorchis* Luhman, 1935 from this same specimen of turtle were reported by Luhman (1935). The first of the new trematodes was from the ventricle of the heart together with *H. synorchis.* The other species was collected while examining the intestine but it is a blood fluke and probably lived in the abdominal blood vessels. Both species are members of the family Spirorchidae.

Type specimens are deposited in the Helminthological Collection of the United States National Museum, Nos. 37178 and 37177.

Neospirorchis pricei n.sp.

(Figs. 1 and 2)

Description (Based on two complete specimens and portions of two others. Figures 1 and 2 were drawn from a specimen which was later accidentally broken in remounting. Although all pieces are still on a single slide, it was decided to consider this specimen a paratype): Body long and slender, apparently more or less flattened (although probably killed under a coverglass), 6.100 to 8.100 mm. long by 0.360 to 0.540 mm. wide; unspined but with very fine transverse ridges. Oral sucker rudimentary, subterminal, 0.052 to 0.057 mm. in diameter; acetabulum lacking. Esophagus slightly sinuous, thick-walled, 0.32 to 0.34 mm. in length; gland cells surrounding its posterior half. Ceca at first with irregular swellings or outpocketings, soon becoming narrow, uniting a little past midbody; common cecum ending blindly near posterior end of body. Excretory pore terminal; excretory vesicle Y-shaped with short spherical median stem.

Genital pore 0.9 to 1.1 mm. from posterior end of body between cecum and gravid uterus, apparently ventral in position, near midline just to the left of the intestinal cecum. Testis long, compactly and spirally coiled, its anterior end near midbody and somewhat anterior to union of the ceca; its posterior end about ¼ body length from posterior end of body; to the left of the common cecum. Loops of the coiled testis sometimes appear to be distinct branches when the coils are superimposed. Vas deferens short. Cirrus sac slightly sinuous, slender, thick-walled, 0.423 mm. long by 0.076 mm. wide, its wall up to 23 μ thick. External seminal vesicle lacking; internal seminal vesicle a narrow elongated sac 0.224 mm. in length or about half the length of the cirrus sac; cirrus muscular, protrusible, about 0.20 mm. long. Ovary a coiled tube; its anterior end overlaps the posterior portion of the testis slightly; its posterior end is a little posterior to the genital pore. The oviduct arises from the posterior end of the ovary. It is at first thin-walled and coiled but soon becomes sinuous and thicker-walled. It extends posteriorly alongside the intestinal cecum almost to the posterior end of the body where it joins the posterior end of the oötype close to the entrance of the vitelline duct. The length of this portion of the oviduct not counting its curvature is about 0.513 mm. Vitelline follicles small and close together, extensive, extending from bifurcation of the ceca to slightly posterior to genital pore; anterior to the cecal union they are extracecal, intercecal, and ventral but not dorsal to the ceca; posterior to the cecal union they are to the right and ventral to the common cecum. A very long, single yolk duct extends posteriorly along the side of the cecum to join the yolk reservoir not far from the posterior end of the body. Yolk reservoir a large irregularly shaped sac, measuring 0.190 by 0.152 mm., with a short anterior duct connecting with the oötype. Oötype extending anteriorly, surrounded by Mehlis' gland which is approximately the same size as the yolk reservoir. A Laurer's' canal and a seminal receptacle are lacking. Anterior to the oötype a straight tube, which can still be considered the oviduct, extends forward about the same distance as the length

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of Mehlis' gland. It then becomes the thin-walled uterus which immediately forms a single, short, close spiral evident only when not more than a few eggs occur in it. As eggs accumulate in this short spiral it appears as a very characteristic, spherical, egg-filled sac. It leads through a narrow constriction to the anterior portion of the uterus, an elongate almost straight sac which, when egg-filled extends slightly anterior to the genital pore. It contains up to an estimated 200 or more eggs. A metraterm is lacking. Eggs are ovoid, yellowish, without filaments or processes, and measure 36 to 40 by 23 to 24μ.

Discussion: Only one other species is known in the genus *Neospirorchis, N. schistosomatoides* Price, 1934, from the heart of *Chelone mydas,* a marine turtle, which died in the National Zoological Park. *N. pricei* differs from this species in possessing (1) a shorter esophagus; (2) more anterior extent of testis; (3) longer, more slender, thicker-walled cirrus sac; (4) a short, inconspicuous vas deferens; (5) a distinct, tightly spiraled posterior portion of the uterus separated from an anterior sac-like portion; (6) ventral genital pore. The seemingly ventral position of the genital pore could possibly be due to compression of the worm when killed under a cover glass. However, there is no evidence of a half twist in the body.

The species is named in honor of Dr. E. W. Price.

Host: Caretta caretta, loggerhead turtle

Location: ventricle of the heart

Locality: Tortugas, Florida

Carettacola bipora n.gen., n.sp.

(Figs. 3–8)

Description (Based on one complete specimen and the anterior two-thirds of another): Body elongate, 4.51 by 0.34 mm., more or less flattened, of almost uniform width. Oral sucker terminal; small, short spines around the mouth and scattered within the oral cavity. Acetabulum located 0.93 mm. from the anterior end of the body, immediately posterior to the intestinal bifurcation. It is weakly muscular, protrusible, more or less leaf-like and membraneous. When retracted, it appears in ventral view (Fig. 4) to possess an outer portion in the form of a thin lobe with short, rather thick, pointed spines along its edge. When protruded, the acetabulum (Fig. 5) flares out into a thin disc with a spined, somewhat convoluted outer edge. Near the center of this flaplike fold is a small ovoid aperture, about 27 by 11 μ in diameter, surrounded by weakly developed circular muscles and very few inconspicuous radial muscles. This pore is very inconspicuous and leads to no visible cavity when the acetabulum is expanded (Fig. 5) although it appears to overlie a transverse cavity when the acetabulum is contracted (Fig. 4). The acetabulum appears to be a flexible, rather membranous structure, very changeable in form. In the contracted state (Fig. 4) it measured about 0.201 mm. in transverse diameter and only about 0.121 mm. in length. In the protruded state it had a length of about 0.247 mm. with a width undetermined because the left portion was folded under.

Esophagus long, sinuous, thick-walled; divided into a long, gradually widening anterior portion and a shorter, flask-shaped portion which is at first narrow then expands abruptly. The ceca originate from the lateral sides of the posterior portion. In the complete (4.51 mm.) specimen the anterior portion of the esophagus measures 0.339 mm., the posterior portion 0.138 mm. in length; total length 0.477 mm. Gland cells occur along the esophagus particularly just posterior to the oral sucker and around the narrow part of the flask-shaped portion. The intestinal ceca are only slightly sinuous and end near the anterior end of the forks of the excretory vesicle not far from the posterior end of the body.

The excretory vesicle is Y-shaped, the median stem measuring 0.13 mm. and each of the two forks about 0.14 mm. The excretory pore is terminal.

The genital pores are ventral, 0.891 mm. (or 1/5 total body length) from the anterior end; 0.17 mm. posterior to the acetabulum. The conspicuous, muscular uterine pore is about halfway from midline to left edge of the body; the inconspicuous male pore closely median to it. The testes are arranged in a linear series of about 37 filling the intercecal area in the posterior 3/5 of the body. The posterior testis is 0.385 mm. from posterior end of body. The external seminal vesicle is sac-like, wider than long, 0.154 mm. wide by 0.077 mm. long, anterior to genital pore, anterior to cirrus sac, filling the intercecal area between acetabulum and cirrus sac. The cirrus sac is large, 0.40 mm. long by 0.16 mm. wide, partly anterior but mostly posterior to genital pores, partly overlapping ovary, containing a tubular seminal vesicle which extends backward then bends abruptly forward toward the genital pore, numerous large prostatic cells, and a short cirrus.

Ovary elongate-ovoid, intercecal, about 1/3 body length from anterior end, 0.315 mm. long by 0.154 mm. in greatest width. The oviduct extends posteriorly, is at first somewhat sinuous then turns to the right and enters the small spherical seminal receptacle. A continuation of the

oviduct extends straight to the right then at the edge of the right cecum bends directly back upon itself and, dorsal to the seminal receptacle, receives connection with a very large, ovoid vagina. This vagina (?) opens on the right side of the body through a conspicuous pore located 1.40 mm. from the anterior end of the body, opposite the posterior end of the ovary. The organ extends diagonally inwardly and posteriorly and measures 0.246 by 0.123 mm., or almost as large as the ovary. Most of the organ is filled by granular cells which radiate toward the center appearing more or less filamentous. Granules near the periphery are especially evident. Near the pore, there is a short region which seems to have striae or folds. The posterior end of the sac narrows abruptly and joins the oviduct which is here a rather wide tube. The radially fibrous appearance of the "vagina" continues in the wall of the oviduct for a short distance. This rather wide portion of the female tube crosses the early oviduct ventrally and extends anteriorly along the left side of the cirrus sac to the muscular uterine pore. Perhaps it should not be considered a uterus until it receives the vitelline duct about 0.2 mm. from the genital pore. The vitelline follicles extend, chiefly extracecal, from the level of the anterior testis to the tips of the ceca. The right vitelline duct crosses the basal portion of the vagina ventrally and joins the left duct near the posterior end of the ovary. A common vitelline duct extends anteriorly ventral to the oviduct which it joins 0.2 mm. posterior to the uterine pore. The true uterus is thus very short. In one specimen it contained a single egg. The egg is thin-shelled, ovoid, 60 by 32 μ, and has a single, posterior, short filament bent slightly at its tip. A Mehlis' gland and Laurer's canal could not be seen.

Host: Caretta caretta, the loggerhead turtle

Location: Found in washings of the intestine, probably originally from some blood vessel.

Locality: Tortugas, Florida

Discussion: Carettacola is clearly a member of the family Spirorchidae, and is probably most closely related to *Vasotrema, Hapalotrema,* or *Hapalorhynchus.* A membraneous acetabulum apparently also occurs in the genus *Leardius* which is entirely different in other respects, notably its preovarian testes and posterior genital pore. *Carettacola* differs from all known blood flukes in possessing a very large vagina-like structure opening laterally posterior to the ovary. In fact, all digenetic trematodes supposedly lack a vagina separate from a metraterm. The significance of this organ is not understood. It is probably at least partly glandular since evident granules occurred in the basal portion of its cells. Near the pore is a short, thick-walled portion. The organ narrows abruptly to open into the oviduct. Thus, it has a position corresponding to Laurer's canal. It differs from a typical Laurer's canal in that it is very large and glandular and opens laterally rather than dorsally. Possibly it could be compared with the small uterine pouch described by Byrd (1939) in *Unicaecum dissimilis* Byrd, 1939. In addition to possessing this distinctive organ, *Carettacola* differs from *Hapalorhynchus* in possessing numerous, rather than two, testes which are not separated by the ovary; in its ventral genital pore; and its well-developed cirrus sac. It differs from *Hapalotrema* in that the testes are not separated into two groups by the ovary, the genital pore is more anterior, and the cirrus sac better developed. *Carettacola* resembles *Vasotrema* except the testes are separated rather than fused into a single spiral testis. However, connections between testes are sometimes difficult to observe in toto-mount, and *Vasotrema* is perhaps the most closely related genus.

The name *"Carettacola"* indicates an inhabitant of *Caretta;* the name *bipora* refers to the uterine pore and the lateral pore of the vagina-like organ.

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Explanation of Plate

All figures, except Fig. 7, were made with the aid of a camera lucida. The value of each scale is in mms.

Abbreviations

ac	acetabulum
cs	cirrus sac
es	esophagus
esv	external seminal vesicle
gp	genital pore
i	intestinal cecum
mg	Mehlis' gland
od	oviduct
ov	ovary
sr	seminal receptacle
t	testis
up	uterine pore
ut	uterus
v	vagina-like organ
vt	vitellaria
yd	yolk duct
yr	yolk reservoir

Fig. 1. *Neospirorchis pricei.* Dorsal view.

Fig. 2. *N. pricei.* Enlarged view of posterior portion of the body. Dorsal view.

Fig. 3. *Carettacola bipora.* Ventral view.

Fig. 4. Retracted acetabulum of *C. bipora.* Ventral view.

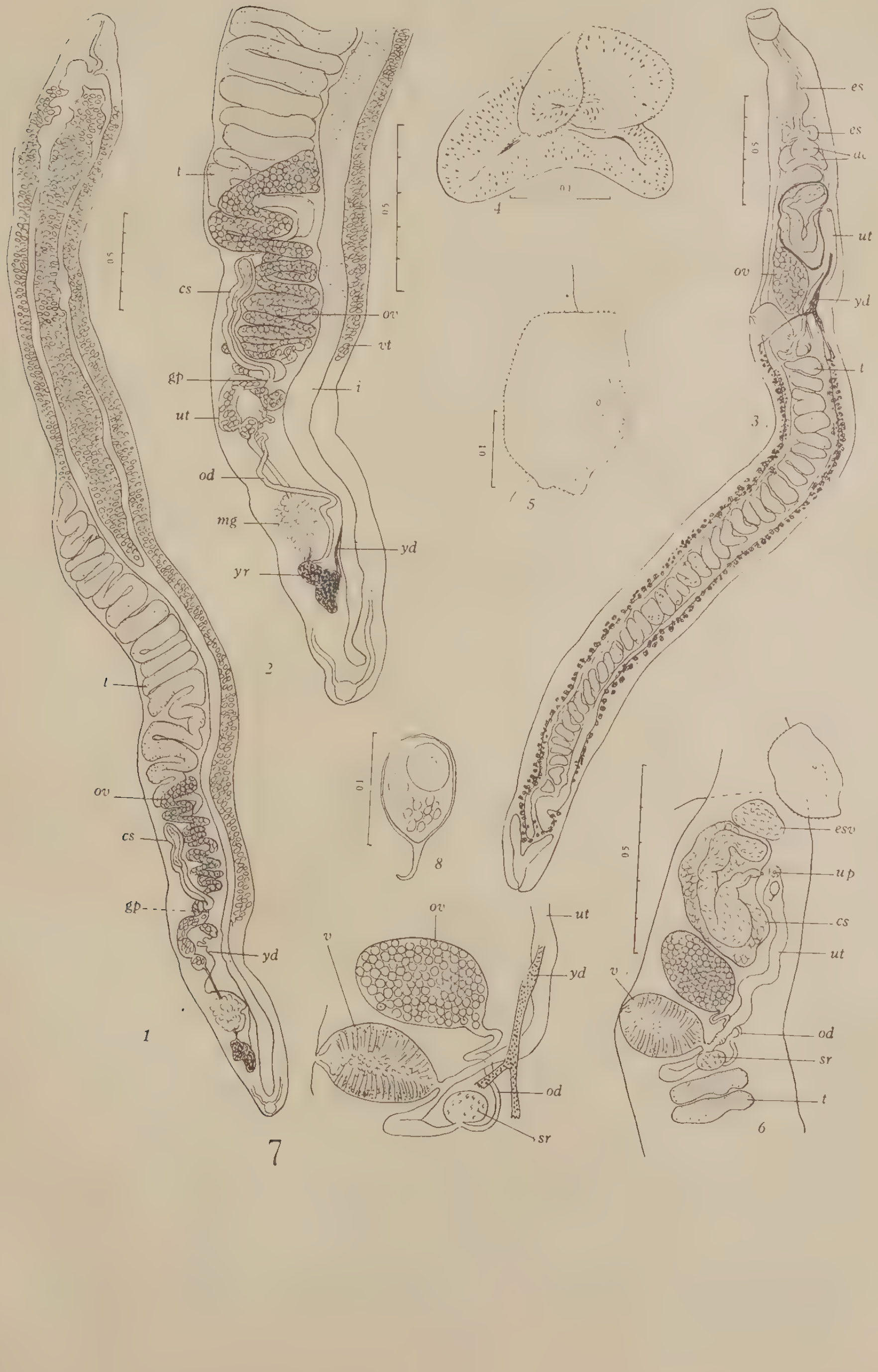
Fig. 5. Protruded acetabulum of *C. bipora.* Semi-ventral view.

Fig. 6. Reproductive organs of *C. bipora.* Ventral view.

Fig. 7. Diagram of female reproductive organs of *C. bipora* showing connections of the vagina-like organ. Ventral view.

Fig. 8. Egg of *C. bipora.*

PLATE I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